A

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Appeal Brief OK

TIME : 10/29/2006 00:45
NAME : DR NIDA & ZUHAIR
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FAX NO./NAME	015712730053
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PAGE(S)	17
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TRANSMISSION VERIFICATION REPORT

Appeal Brief correction of date

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FAX NO./NAME	015712730053
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Board of Appeal and Interferences
USPTO
Fax No.:+ 571-273-0053

Appeal Brief submitted further to

Notice of Appeal from the Examiner to the Board of Appeal and Interferences

Submitted in Response to Office Action dated 07/11/2006 (Final Rejection)

Date: December 10, 2006

Correction of fax date

The date shown on the fax letter submitted December 10th 2006 is incorrect because the fax machine setting is wrong.

The date shown in my Transmission Verification Report is 10/29/2006 00:28

Actual date is 12/10/2006, local time in Doha-Qatar is 10 PM

Thank you for your effort and excuse my limitations.

Best regards

Sincerely yours

N. Nassief

Nida Nassief

(2)

UNITED STATES PATENT AND TRADEMARK OFFICE

COMMISSIONER FOR PATENTS
UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
www.uspto.gov

APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY.DOCKET.NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/944,564	09/04/2001	1623	1259			24	15

CONFIRMATION NO. 8476

UPDATED FILING RECEIPT

OC000000007223833

AL-JASSIM, Rawaa
2578 River Woods Drive
Naperville, IL 60565

Date Mailed: 12/21/2001

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Customer Service Center. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Nida Abdul-Ghani Nassief, Doha, IRAQ;

Domestic Priority data as claimed by applicant

Foreign Applications

UNITED KINGDOM 9904777.1 03/02/1999

If Required, Foreign Filing License Granted 10/06/2001

Projected Publication Date: 03/28/2002

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Asthma/allergy therapy that targets T-lymphocytes and/or eosinophils

Preliminary Class

514

Board of Appeal and Interferences
USPTO
Fax No.:+ 571-273-0053

Appeal Brief submitted further to

Notice of Appeal from the Examiner to the Board of Appeal and Interferences

Submitted in Response to Office Action dated 07/11/2006 (Final Rejection)

Date: December 09, 2006

A- Identification page

Applicant name: Nida Nassief

Application number: 09/944,564

Filling Date of the application: 09/04/2001

Title of the invention: Asthma/allergy therapy that targets T-lymphocytes and/or eosinophils.

The name of the examiner: Patrick T. Lewis

Art unit of the examiner: 1623

Title of the paper: **Appeal Brief**

N. Nassief
Signature
12-10-2006

B- Table of contents

B- Table of contents

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Annexes

B. appeal
C. Sanchez publication
D G.B. priority Document
E References from appeal
F Examination report Aug. 15, 04

Dear Examiner:

Further to the appeal submitted to the Board of Appeal and Interference dated Oct 10th 2006, please find following the Appeal Brief in compliance with 37 CFR 41.37.

I am dissatisfied with the primary examiner's decision of final rejection dated 07/11/2006 of my claim number 25

C- Real party in interest

I am a self supported 57 years old female medical doctor, the applicant, owner, pro se inventor of the US patent application number: 09/944,564.

D- Related appeal and interferences

None, this is my first appeal.

E- Status of claims

A statement of the status of all the claims in the proceeding (e.g., rejected, allowed or confirmed, withdrawn, objected to, canceled) and an identification of those claims that are being appealed is in the following 3 pages.

Pages 6-7: indicate claimed as filed 07-15-2003

Page 8: indicate amended claims as filed 10-24-2003

Claim pages

CLAIMS

1. (Currently amended) Use of glycophosphopeptical for the treatment ~~and/or prophylaxis~~ of allergy/asthma for administration to a mammal ~~such as a~~ human in need of such treatment.

2. (Withdrawn)

3. (Original) A Pharmaceutical composition comprises glycophosphopeptical, in any pharmacologically active form at a concentration of the extract which is effective as a Th1 stimulating agent.

4. A Pharmaceutical composition as claimed in claim 3 further comprising an excipient.

5. A method of treatment of diseases caused by type I IgE-mediated hypersensitivity reaction comprising the administration to a mammal such as a human in need of such treatment, of an effective dose of glycophosphopeptical.

6. (Currently amended) The claim 4 including a dosage regimen as a characterizing feature, administering to a patient suffering from a chronic disease a short-term therapy of 5-20 days, ~~preferably 5 days,~~ of a Th1 stimulating agent, to get a long-term clinical remission of months as a result of selective switching-off of the eosinophilic inflammation.

7. The use of the pure seeds of Nigella sativa for the preparation of an asthma and allergy agent in a concentration which was found to perform substantially the same function in substantially the same way to obtain substantially the same results as with glycophosphopeptical.

8. A Pharmaceutical composition as claimed in claim 6 further comprising an excipient.

9. A medicament as claimed in any preceding claim, which is adapted and/or packaged for periodic administration to said mammal in doses over a period of 5-20 days, preferably 5 days in doses at least once daily up to ten times/day.

10. A medicament as claimed in claim 9, characterized in that each one of said doses comprises up to 2000mgs of said active agent, preferably about 200-1000mgs, of said active agent , adapted for oral administration to said mammal in capsules, or tablets, or lozenges, or as a powder, or a suspension, or a syrup

11. (Withdrawn).

12. A kit comprising a medicament as claimed in claim 10 and 11 packaged in separate doses for periodic administration to said mammal such as a human, contains written or printed instructions.

13. The method of claim 5 and 7 is dependent on the fact that interferon is an in vivo Eosinophilic Chemotactic Factor, and that serum interferon and Th1 lymphocytes are controlling the pre-inflammatory phase of allergic reaction.

14. (Withdrawn).

15. The method of claim 5 and 7 wherein the recommended dose of Th1 lymphocytes stimulating agent is sufficient to selectively switch –off th eosinophilic inflammation in the patient's airway.

16. The method of claim 5 and 6 wherein Th1 lymphocytes stimulating agents, are capable of stimulating T lymphocytes in culture, comparable to Purified Protein Derivative of BCG, as a classical Cell Mediated Immunity stimulating agent.

17. (Withdrawn)

18. A method of treatment of viral respiratory tract infections ~~such as, but not limited to~~ influenza and common cold, ~~other viral infections~~ comprising the administration to a mammal such as a human in need of such treatment, of an effective dose of Th1 stimulating agents.

19. (Withdrawn).

20. (Withdrawn).

21. A method of treatment of crohn's disease comprising the administration to a mammal such as a human in need of such treatment, of an effective dose of Th1 stimulating agents in order to stimulate Cell Mediated Immunity.

22. Use of Th1 stimulating agent, for the treatment of crohn's disease to be administered to a mammal such as a human in need of such treatment.

23. (Withdrawn).

24. (Withdrawn).

CLAIMS

1 – 24. (Withdrawn)

25. (New) A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of allergy and asthma in dosage and duration which is effective to:

i- Switch-off the airway eosinophilic inflammation.

ii- Reduce mucus secretion.

iii- Reduce symptom scores significantly.

iv- Restore airways patency as measured by Pulmonary Function Test.

26. (New) A pharmaceutical composition of claim 25, to induce a clinical remission and long-term therapeutic effect in a chronically ill patient.

27. (New) A method of treatment of allergy and asthma patients in need of multiple drugs daily, comprising of administering the pharmaceutical composition of claim 25 for a short course of 1-14 days to induce a remission of 3-12 months.

28. (New) A pharmaceutical composition for the treatment of allergy and asthma consisting essentially of the herbal seeds of Nigella sativa to act as a vaccine that is almost identical to Purified Protein Derivative from Bacillus Calmette Guréin:

i- Switch-off the airway eosinophilic inflammation.

ii- Reduce mucus secretion.

iii- Reduce symptom scores significantly.

iv- Restore airways patency as measured by Pulmonary Function Test.

29. (New) A pharmaceutical composition vaccine of claim 28, to induce a clinical remission and long-term therapeutic effect in a chronically ill patient suffering from asthma and allergy.

30. (New) A method of treatment of allergy and asthma patients in need of multiple drugs daily, comprising of administering the pharmaceutical composition of claim 28 for a short course of 1-14 days to induce a remission of 3-12 months.

31. (New) A pharmaceutical composition vaccine from Nigella sativa, to induce a clinical remission and long-term therapeutic effect in patients with Crohn's disease.

32. (New) A method of treatment of Crohn's disease, comprising of administering the pharmaceutical composition vaccine from Nigella sativa for a short course of 1-14 days to induce symptomatic remission.

33. (New) A pharmaceutical composition vaccine from Nigella sativa, to induce a clinical remission and long-term therapeutic effect in patients with influenza and common cold.

34. (New) A method of treatment of influenza and common cold, comprising of administering the pharmaceutical composition vaccine from Nigella sativa for a short course of 1-14 days to induce symptomatic remission.

F- Status of amendments

Status of any amendment filed subsequent to final rejection is as follows:

- The amendment was entered? Reply Yes, date 10-10-2006

- The amendment has been acted upon by the examiner? Reply: No

G- Summary of claimed subject matter

The claimed invention is related to a pharmaceutical composition consisting essentially of glycophosphopeptical that is used for the treatment of allergy and asthma in claim 25 of the patent.

I will separate the subject matter related to asthma from subject matter related to allergy; because in the Examiner Office Action dated 07/11/2006 the use in asthma have been rejected and the use in allergy have been overlooked and forgotten.

For each of the 2 claimed inventions involved in the appeal, I shall refer to the specification by page and line number, and the best method for carrying out the invention (enablement) and results of early clinical testing including: 1- X-ray films, 2- Photographs of microscopical examination of sputum and 3- statistical analysis of improvement in symptom scores.

Please notice that in the Priority Document I have referred to glycophosphopeptical as substance A or drug N as clarified in page 1 line 3 of the priority document.

Asthma

It appears in the patent application in the following pages:

		From		To	
		Page	Line	Page	Line
Patent Application					
	Disclosure of the Invention	7	1	7	12
		10	13	10	19
	Best Mode of Carrying the Invention	11	2	17	3
Priority Document	Advantages	3			
	Indications for the use of substance A	2	7		
	Advantages	3	Full page		
	Abstract	15	8 lines from the abstract		End of the page
	Stage III	19	Last paragraphs	31	Full page
	Stage IV Including photographs	33	Lower half	37	

	of microscopical exam sputum				
Response to Office action dated May 5, 2004 filed August 4, 2004					
	Table of comparison	9	Full page		
	Results of clinical studies	10	Full page	11	Upper half table
	Reduction in Sputum eosinophil graph	20	Full page		

Allergy including allergic rhinitis and rhinosinusitis

		From		To	
		Page	Line	Page	Line
Patent Application					
	Disclosure of the Invention	7	1	7	12
		10	13	10	19
	Best Mode of Carrying the Invention	11	2	17	3
Priority Document					
	Indications for the use of substance A	2	7	2	10
	Abstract	15	First 8 lines from yhe abstract		
	Stage II	17	Last 2 paragraphs	19	Upper half until stage III
	Table of outcome clinical testing OTHER ALLERGIES including allergic conjunctivitis, chronic urticaria, laryngeal oedema	18	Full page		
Response to Office action dated May 5, 2004 filed August 4, 2004					
		7	Full page		
	Results of clinical studies allergic rhinitis	11	Lower half of the page	15	
	X ray photograph	16	Full page		
	Statistical analysis	18	Full page	19	Full page

H- Grounds of rejection to be reviewed on appeal

It is a single ground of rejection related to prior art.

In the Office Action dated 07/11/2006 the Examiner have rejected claim 25 – 27, in pages 3 – 4 point number 7 reads as: Claims 25-27 are rejected under 35 U. S. C. 102(b) as being anticipated by the following prior art:

" Valoracion clinica immunologica de un modificador de la respuesta biologica, AM3, en el tratamiento de la patologia respiratoria infectiosa infantile", Sanchez Palacios A. et. al. Allergol Immunopathos (Madr) (1992), Vol 20 (1), pages 35-39 (Sanchez).

Sanchez discloses the use of Immunoferon (AM3) in the treatment of childhood infectious respiratory pathology. To assess the immunoclinical effectiveness of a biologic response immunomodulator, glycophosphopeptide (AM3) was administered to 20 children with asthmatic bronchitis. The children received 2 envelops (1gm) daily for 4 months. The clinical and immunological parameters assessed were: cough, dyspnoea, expectoration, frequency and intensity of bronchospasm, time of administration of the symptomatic medication, and the delayed cutaneious cells response by means of the intradermal reaction of 5 antigens: Immunoferon reduced the symptoms, the intensity and frequency of the bronchospasm, and the symptomatic medication.

Point number 8 reads as: Aplicant's argument filed April 12, 2006 has been fully considered but they are not persuasive. Applicant's argue that Sanchez is referring to infectious respiratory pathology (asthmatic bronchitis) which is not bronchial asthma which is allergic or atopic.

Applicant's arguments have been considered but are not deemed germane. Sanchez teaches the use of glycophosphopeptide for treating asthmatic bronchitis. It was well known in the art at the time of the invention that asthmatic bronchitis is a condition in which the airways in the lung are obstructed due to both persistent asthma and bronchitis. Thus, the patient population treated by the method of Sanchez embraces asthma patients and therefore meets the limitation of the instantly claimed invention.

I- Argument

The following argument have been filed dated October 10, 2006 and is entered in the patent application report. It includes a copied chapter from a medical textbook that will help very much in clarifying the dispute.

Appeal filed October 10, 2006 page 2 reads as:

In this appeal Brief, may I kindly request the Board of Appeal to consider the following two requests:

First: I am still arguing that asthma and asthmatic bronchitis are two separate unrelated diseases and that my claim rejection was brought up by confusion in the name between the old term of asthmatic bronchitis and asthma. Accordingly the use of glycophosphopeptical in the treatment of asthma in my patent is novel and kindly requesting its allowance.

Second: Claim 25 reads as "A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of allergy and asthma ....etc", I am arguing that the pharmaceutical composition for the treatment of "allergy" as a group of diseases referred to separately in the patent application, under description of the invention, with enabelment and previous clarification in my reply to the Office Action filed on Aug 2004 with X-ray films clarifying its unique outcome of early clinical testing , and will be detailed later, have been forgotten and overlooked. May I kindly request the allowance of this claimed invention.

Asthma is currently an international enigma with increasing incidence and uncontrolled patients. According to medical reports released during 2006 from the "Global Initiative Of Asthma" that will be included in the mail copy of this Response and Appeal.

Appeal filed October 10, 2006Last paragraph page 2 – 2 paragraphs from page 3) reads as:

My argument filed April 12, 2006 was that the Sanchez had excluded cases of asthma in patients selection as described in page 36 column 1 of the article as follows:

"MATERIAL Y METODOS

Pacientes. Se seleccionaron 40 ninos **no atopicos** con clinica respirotoria infecciosa de bronquuitis espastica y/o asmatica con pruebas **cutaneas a neumoallergenos negative** e **IgE total normal.**

May I **add** to my argument filed April 12, 2006 that referring to the title of the article by Sanchez which reads as " Valoracion clinica immunologica de un modificador de la respuesta biologica, AM3, en el tratamiento de la **patologia respiratoria infectiosa infantile",** this description fits the condition of asthmatic bronchitis "bronchiolitis" as will follow, but not asthma.

In the Examiners Office Action dated 07/11/2006, page 4, line 7, he have the following comment "It was well known in the art at the time of the invention that asthmatic bronchitis is a condition in which the airways in the lungs are obstructed due to both persistent asthma and bronchitis."

In the Examiners Office Action dated 07/11/2006, page 4, line 7, he have the following comment "It was well known in the art at the time of the invention that asthmatic bronchitis is a condition in which the airways in the lungs are obstructed due to both persistent asthma and bronchitis."

My reply is that "The term bronchiolitis was first used by Engle and Newns in 1940, bronchiolitis appears to have been born from a long lineage of confusing sobriquets, including "asthmatic bronchitis." As will be described below under the title "Bronchiolitis" page 5 of this report. Therefore at the time of filing my invention it was well known that asthma and asthmatic bronchitis are two separate diseases.

Bronchiolitis (Asthmatic Bronchitis) (page 4 appeal filed October 10, 2006)

Exact definition of asthmatic bronchitis is available from a textbook of "Principles and Practice of Infectious Diseases", selected paragraphs follows indicates that we are dealing with two separate diseases that may coexist in an infant, The following statements contitute a reply to the point raised by the examiner::

Page 812, coloumn 2: "Bronchiolitis is an acute viral lower respiratory tract illness that occurs during the first 2 years of life. **The illness also has been called "wheezy bronchitis" and "asthmatic bronchitis". Whatever term is applied, the syndrome is caused primarily by viral infections. The characteristic clinical manifestations include an acute onset of wheezing and hyperinflation, most commonly associated with cough,** rhinorrhea, tachypnoea (increased respiratory rate) and respiratory distress."

"The term bronchiolitis appears to have been born from a long lineage of confusing sobriquets, including "acute catarrhal bronchitis," "interstitial bronchopneumonia," "spastic bronchopneumonia," "capillary or obstructive bronchiolitis," and "asthmatic bronchitis." Bronchiolitis, however, did not become recognized as a distinct entity until the 1940s."

In page 814, coloumn 1 under the term Pathophysiology **"The term bronchiolitis was first used by Engle and Newns in 1940** for the lower respiratory tract disease observed in young infants that tend to be sever and often fatal. The virus initially replicates in the epithelium of the upper respiratory tract, but in the young infant it tend to spread rapidly to the lower tract airways."

"Inflammatory changes of various severity are observed in most small bronchi and bronchioles. The inflammation and edema make the small-lumen airways in infants particularly vulnerable to obstruction. Thus, although airflow is impended during both inspiration and expiration, the latter is more affected and prolonged."

In the first column, last paragraph in page 815, under the title of "Pathophysiology": "Clarifying the relationship between bronchiolitis and subsequent asthma is complicated by confusion about the Pathophysiology of asthma itself".....Nevertheless, **"The association between bronchiolitis and asthma is not straightforward. Several investigatorshave demonstrated that children with bronchiolitis in infancy have no increased risk for asthma** or abnormal pulmonary function by the time they reach early adolescence.

In the first column of page 816 under the title "Diagnosis" and its continuation in the second column in the same page: "The diagnosis of bronchiolitis is made most frequently on the basis of the characteristic clinical and epidemiological findings. However considerable confusions exist over the exact definition of bronchiolitis. A variety of entities may cause a similar picture of dyspnoea and wheezing in the infant. Asthma is not easily differentiated, particularly if it is the infant's first episode. **Furthermore the two diseases may be combined."**

Annex II1. Caroline Breese Hall and John T. McBride. Bronchiolitis. Chapter 60: 812-819. PRINCIPLES AND PRACTICE OF INFECTIOUS DISEASES. Sixth Edition 2005. Elsevier Churchill Livingstone.

Appeal filed October 10, 2006 page 2 paragraph 3 and down - page 3) reads as:

Most important, to support my argument further, I am submitting new evidence from the standard teaching of medical textbooks that clarifies the point that asthma previously was used to indicate "shortness of breath" as in the case of the term "**cardiac asthma**" that is used to denote shortness of breath in heart failure (Annex II). Furthermore the correlation between asthma and asthmatic bronchitis; selected from the textbook of Principles and Practice of Infectious Diseases 2005 (Annex III), asthmatic bronchitis is currently named bronchiolitis. The term bronchiolitis was first used by Engle and Newns in 1940 for the lower respiratory tract disease observed in young infants. The term bronchiolitis appears to have been born from a long lineage of confusing sobriquets, including "acute catarrhal bronchitis," "interstitial bronchopneumonia," "spastic bronchopneumonia," "capillary or obstructive bronchiolitis," and "asthmatic bronchitis." And that "We are dealing with two separate diseases that may coexist in an infant, and that children with bronchiolitis in infancy have no increased risk of asthma by the time they reach adolescence." This will be detailed further in the following text. May I kindly request consideration of this new evidence and other reference in the text and allow my claimed invention.

Confusing medical terms using asthma

The term asthma, historically, is used to designate any disease characterized by "asthma-like symptoms", in patients complaining of dyspnoea, wheeze, cough and sputum. Those diseases are unrelated to the disease entity of current asthma; examples are 1- "cardiac asthma" and 2- "asthmatic bronchitis".

1- Cardiac Asthma

The clinical manifestations of heart failure includes respiratory disturbances as dyspnoea and paroxysmal nocturnal dyspnoea; this term refers to attacks of sever shortness of breath and coughing that generally occur at night. Cardiac asthma is closely related to paroxysmal nocturnal dyspnoea and nocturnal cough and is characterized by wheezing secondary to bronchospasm-most prominent at night.

Annex II - Part VIII Disorders of the Cardiovascular System: page 1370. HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. 16th Edition (2005) Mc Graw-Hill

J- Claim appendix

In the Office Action dated 07/11/2006 the Examiner have rejected claim 25 - 27 of my patent application number 09/944,564. Currently I am defending claim 25 that reads as follows:

25. A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of allergy and asthma in dosage and duration which is effective to:

i- Switch-off the airway eosinophilic inflammation.

ii- Reduce mucus secretion.

iii- Reduce symptom score significantly.

iv- Restore airway patency as measured by Pulmonary Function test.

K- Evidence appendix

An appendix containing copies of any evidence submitted pursuant to $$ 1.130, 1.131 or 1.132 of this title or any other evidence entered by the examiner and relied upon by appellant in the appeal, along with a statement setting forth where in the record that evidence was entered in the record by the examiner. Reference to unentered evidence is not permitted in the brief. See $ 41.33 for treatment of evidence submitted after appeal. This appendix may also include copies of the evidence relied upon by the examiner as to grounds of rejection to be reviewed on appeal.

1- " Valoracion clinica immunologica de un modificador de la respuesta biologica, AM3, en el tratamiento de la patologia respiratoria infectiosa infantile", Sanchez Palacios A. et. al. Allergol Immunopathos (Madr) (1992), Vol 20 (1), pages 35-39 (Sanchez).

2- Annex II - Part VIII Disorders of the Cardiovascular System: page 1370. HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. 16th Edition (2005) Mc Graw-Hill

3- Annex II1. Caroline Breese Hall and John T. McBride. Bronchiolitis. Chapter 60: 812-819. PRINCIPLES AND PRACTICE OF INFECTIOUS DISEASES. Sixth Edition 2005. Elsevier Churchill Livingstone.

4- Annex 1

L- Related proceedings appendix

None

This is the first appeal submitted

Evidence will be sent by mail

END OF REPORT

B

Appeal
Send second
11/10/06 .3 pages date correction

TRANSMISSION VERIFICATION REPORT

TIME : 10/11/2006 [illegible]:35
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DATE,TIME	10/11 04:33
FAX NO./NAME	015712738300
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TRANSMISSION VERIFICATION REPORT

Appeal
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11/10/06
Appeal
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TEL : 0974-4650664

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PAGE(S)	10
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MODE	STANDARD

TRANSMISSION VERIFICATION REPORT

Appeal number from list

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NAME : DR NIDA & ZUHAIR
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DATE,TIME	10/11 04:55
FAX NO./NAME	015712730053
DURATION	00:06:47

U. S. Patent and Trademark Office
The Examiner
Patrick T. Lewis Ph D
Fax No:+571-273-~~8661~~ 8300

My Reference: Patent Application Number: 09/944,564

Date: October 10, 2006
Total number of pages: 8+ Filing Reciept
Annexes will be sent by mail

Fax letter

Response to Office Action dated 07/11/2006 (Final Rejection)
Notice of Appeal from the Examiner to the Board of Appeal and Interferences

Dear Sir:

In response to the above-identified final rejection of my patent application claims 25-27, may I kindly submit the following response:

1- By submitting a Notice of Appeal From the Examiner to the Board of Appeal and Interferences [Form: PTO/SB/31 (07-06)].
2- Payment by Credit Card. Form PTO-2038 is attached.
3- Objection to the Examiner decision by submitting further evidence from medical textbooks clarifying the point of dispute with the examiner; in relation to the definition of asthma and asthmatic bronchitis, and differentiating them as unrelated separate medical entities, thus rendering my claims in the use of glycophosphopeptical in the treatment of asthma patentable and valid.
4- Amendment of claim 25, currently reads as "25. (New) A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of **allergy** and **asthma** in dosage and duration which is effective to....etc"; may I kindly request separation of the use of glycophosphopeptical in the treatment of allergy from its use in the treatment of asthma. Discussion of the use in the treatment of allergy are part of the response to Office Action dated ....

Detailed response will follow.

Thank you for considering my appeal.

Best regards

N. Nasif

The Inventor
Nida Nasif

Notice of Appeal from the Examiner to the Board of Appeal and Interferences

Submitted in Response to Office Action dated 07/11/2006 (Final Rejection)

Date: October 10, 2006

Dear Sir:

Please fid following detailed response:

Rejection of Record Set Forth in the Office Action Dated November 21,2005

In this Office Action the Examiner have rejected claim 25 - 27 of my patent application number 09/944,564. Currently I am defending claim 25 that reads as follows:

25. (New) A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of **allergy** and **asthma** in dosage and duration which is effective to:

i- Switch-off the airway eosinophilic inflammation.
ii- Reduce mucus secretion.
iii- Reduce symptom score significantly.
iv- Restore airway patency as measured by Pulmonary Function Test.

The cause of rejection

From the above identified Office Action pages 3 - 4:

7. Claims 25-27 are rejected under 35 U. S. C. 102(b) as being anticipated by Sanchez Palacios A. et. al. Allergol Immunopathos (Madr) (1992), Vol 20 (1), pages 35-39 (Sanchez).

Sanchez discloses the use of Immunoferon (AM3) in the treatment of childhood infectious respiratory pathology. To assess the immunoclinical effectiveness of a biologic response immunomodulator, glycophosphopeptide (AM3) was administered to 20 children with asthmatic bronchitis. The children received 2 envelops (1gm) daily for 4 months. The clinical and immunological parameters assessed were: cough, dyspnoea, expectoration, frequency and intensity of bronchospasm, time of administration of the symptomatic medication, and the delayed cutaneious cells response by means of the intradermal reaction of 5 antigens: Immunoferon reduced the symptoms, the intensity and frequency of the bronchospasm, and the symptomatic medication.

8. Aplicant's argument filed April 12, 2006 has been fully considered but they are not persuasive. Applicant's argue that Sanchez is referring to infectious respiratory pathology (asthmatic bronchitis) which is not bronchial asthma which is allergic or atopic.

Applicant's arguments have been considered but are not deemed germane. Sanchez teaches the use of glycophosphopeptide for treating asthmatic bronchitis. It was well known in the art at the time of the invention that asthmatic bronchitis is a condition in which the airways in the lung are obstructed due to both persistent asthma and bronchitis. Thus, the patient population treated by the method of Sanchez embraces asthma patients and therefore meets the limitation of the instantly claimed invention.

Appeal From the Examiner to the Board of Appeal and Interferences

What is my dispute difference of opinion) with the Examiner in relation to Office Action dated 07/11/2006

In this appeal, may I kindly request the Board of Appeal to consider the following two requests:

First: I am still arguing that asthma and asthmatic bronchitis are two separate unrelated diseases and that my claim rejection was brought up by confusion in the name between the old term of asthmatic bronchitis and asthma. Accordingly the use of glycophosphopeptical in the treatment of asthma in my patent is novel and kindly requesting its allowance.

Second: Claim 25 reads as "A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of allergy and asthma ....etc", I am arguing that the pharmaceutical composition for the treatment of "allergy" as a group of diseases referred to separately in the patent application, under description of the invention, with enabelment and previous clarification in my reply to the Office Action filed on Aug 2004 with X-ray films clarifying its unique outcome of early clinical testing , and will be detailed later, have been forgotten and overlooked. May I kindly request the allowance of this claimed invention.

In this reply, references to the standard teaching of medical textbooks are made for detailed description of asthmatic bronchitis. Selected chapters are photocopied, and the relevant paragraphs are underlined in order to clarify the source of confusion in the name, the differentiating clinical features, and the correlation between asthmatic bronchitis and asthma. I am trying to keep the text minimal, but excuse me for placing some paragraphs and sentences of secondary importance to keep the continuity of the reply.

Asthma is currently an international enigma with increasing incidence and uncontrolled patients. According to medical reports released during 2006 from the "Global Initiative Of Asthma" that will be included in the mail copy of this Response and Appeal.

Detailed Appeal / First

Claim 25 in relation to "A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of asthma"

My argument filed April 12, 2006 was that the Sanchez had excluded cases of asthma in patients selection as described in page 36 column 1 of the article as follows:

"MATERIAL Y METODOS

Pacientes. Se seleccionaron 40 ninos **no atopicos** con clinica respirotoria infecciosa de bronquuitis espastica y/o asmatica con pruebas **cutaneas a neumoallergenos negative** e **IgE total normal.**

May I **add** to my argument filed April 12, 2006 that referring to the title of the article by Sanchez which reads as " Valoracion clinica immunologica de un modificador de la respuesta biologica, AM3, en el tratamiento de la **patologia respiratoria infectiosa infantile",** this description fits the condition of asthmatic bronchitis "bronchiolitis" as will follow, but not asthma.

In the Examiners Office Action dated 07/11/2006, page 4, line 7, he have the following comment "It was well known in the art at the time of the invention that asthmatic bronchitis is a condition in which the airways in the lungs are obstructed due to both persistent asthma and bronchitis." **My reply** is that "The term bronchiolitis was first used by Engle and Newns in 1940, bronchiolitis appears to have been born from a long lineage of confusing sobriquets, including "asthmatic bronchitis." As will be described below under the title "Bronchiolitis" page 5 of this report. Therefore at the time of filing my invention it was well known that asthma and asthmatic bronchitis are two separate diseases.

Most important, to support my argument further, I am submitting new evidence from the standard teaching of medical textbooks that clarifies the point that asthma previously was used to indicate "shortness of breath" as in the case of the term "**cardiac asthma**" that is used to denote shortness of breath in heart failure (Annex II). Furthermore the correlation between asthma and asthmatic bronchitis; selected from the textbook of Principles and Practice of Infectious Diseases 2005 (Annex III), asthmatic bronchitis is currently named bronchiolitis. The term bronchiolitis was first used by Engle and Newns in 1940 for the lower respiratory tract disease observed in young infants. The term bronchiolitis appears to have been born from a long lineage of confusing sobriquets, including "acute catarrhal bronchitis," "interstitial bronchopneumonia," "spastic bronchopneumonia," "capillary or obstructive bronchiolitis," and "asthmatic bronchitis." And that "We are dealing with two separate diseases that may coexist in an infant, and that children with bronchiolitis in infancy have no increased risk of asthma by the time they reach adolescence." This will be detailed further in the following text. May I kindly request consideration of this new evidence and other reference in the text and allow my claimed invention.

Confusing medical terms using asthma

The term asthma, historically, is used to designate any disease characterized by "asthma-like symptoms", in patients complaining of dyspnoea, wheeze, cough and sputum. Those diseases are unrelated to the disease entity of current asthma; examples are 1- "cardiac asthma" and 2- "asthmatic bronchitis".

1- Cardiac Asthma

The clinical manifestations of heart failure includes respiratory disturbances as dyspnoea and paroxysmal nocturnal dyspnoea; this term refers to attacks of sever shortness of breath and coughing that generally occur at night. Cardiac asthma is closely related to paroxysmal nocturnal dyspnoea and nocturnal cough and is characterized by wheezing secondary to bronchospasm-most prominent at night.

Annex II - Part VIII Disorders of the Cardiovascular System: page 1370. HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. 16th Edition (2005) Mc Graw-Hill

2- **Bronchiolitis (Asthmatic Bronchitis)**

Exact definition of asthmatic bronchitis is available from a textbook of "Principles and Practice of Infectious Diseases", selected paragraphs follows indicates that we are dealing with two separate diseases that may coexist in an infant, The following statements contitute a reply to the point raised by the examiner::

Page 812, coloumn 2: "Bronchiolitis is an acute viral lower respiratory tract illness that occurs during the first 2 years of life. **The illness also has been called "wheezy bronchitis" and "asthmatic bronchitis". Whatever term is applied, the syndrome is caused primarily by viral infections. The characteristic clinical manifestations include an acute onset of wheezing and hyperinflation, most commonly associated with cough,** rhinorrhea, tachypnoea (increased respiratory rate) and respiratory distress."

"The term bronchiolitis appears to have been born from a long lineage of confusing sobriquets, including "acute catarrhal bronchitis," "interstitial bronchopneumonia," "spastic bronchopneumonia," "capillary or obstructive bronchiolitis," and "asthmatic bronchitis." Bronchiolitis, however, did not become recognized as a distinct entity until the 1940s."

In page 814, coloumn 1 under the term Pathophysiology **"The term bronchiolitis was first used by Engle and Newns in 1940** for the lower respiratory tract disease observed in young infants that tend to be sever and often fatal. The virus initially replicates in the epithelium of the upper respiratory tract, but in the young infant it tend to spread rapidly to the lower tract airways."

"Inflammatory changes of various severity are observed in most small bronchi and bronchioles. The inflammation and edema make the small-lumen airways in infants particularly vulnerable to obstruction. Thus, although airflow is impended during both inspiration and expiration, the latter is more affected and prolonged."

In the first column, last paragraph in page 815, under the title of "Pathophysiology": "Clarifying the relationship between bronchiolitis and subsequent asthma is complicated by confusion about the Pathophysiology of asthma itself".....Nevertheless, **"The association between bronchiolitis and asthma is not straightforward. Several investigatorshave demonstrated that children with bronchiolitis in infancy have no increased risk for asthma** or abnormal pulmonary function by the time they reach early adolescence.

In the first column of page 816 under the title "Diagnosis" and its continuation in the second column in the same page: "The diagnosis of bronchiolitis is made most frequently on the basis of the characteristic clinical and epidemiological findings. However considerable confusions exist over the exact definition of bronchiolitis. A variety of entities may cause a similar picture of dyspnoea and wheezing in the infant. Asthma is not easily differentiated, particularly if it is the infant's first episode. **Furthermore the two diseases may be combined."**

Annex II1. Caroline Breese Hall and John T. McBride. Bronchiolitis. Chapter 60: 812-819. PRINCIPLES AND PRACTICE OF INFECTIOUS DISEASES. Sixth Edition 2005. Elsevier Churchill Livingstone.

Asthma – selected paragraphs related to my invention

The problem is related to the diseases manifested clinically by the triad of cough dyspnoea and wheeze.

Definition: Asthma is defined as a chronic inflammatory disease of the airways of any age. The symptoms of asthma consist of a triad of dyspnoea (shortness of breath), cough, and wheezing (respiration becomes audibly harsh and expiration becomes prolonged). The end of an episode is frequently marked by a cough that produces thick stringy mucus, when examined microscopically, often shows eosinophils (P1511). Asthma is an episodic disease, with acute exacerbations interspaced with symptom-free periods. Typically most attacks are lasting minutes to hours spontaneously or after treatment (P1508). The eosinophil appears to play an important part in the infiltrative component (P1509).

Stimuli that incite asthma (provoke acute episode) can be grouped into seven major categories (P1509):

- Allergens in allergic asthma (25-35% of all cases, young age up to 30 years) is dependant on an IgE response controlled by T and B lymphocytes and activated by interaction of antigen with mast cell-bound IgE molecule, mostly inhaled antigens (as pollens, dust, dust mite, cat dander, grasses ... ect).
- pharmacologic,
- environmental,
- occupational,
- infectious: respiratory infections are the most common of the stimuli that evoke acute exacerbation of asthma.
- exercise-related, and
- emotional

Differential Diagnosis of asthma (P1511)

The differentiation of asthma from other diseases associated with dyspnoea and wheezing is usually not difficult, particularly if the patient is seen during an acute episode. The physical findings, symptoms and the history of periodic attacks are quite characteristic. A personal and family history of allergic diseases such as eczema, rhinitis, or urticaria is valuable contributory evidence. An extremely common feature of asthma is nocturnal awakening with dyspnoea that its absence raises doubt about the diagnosis.

Upper airway obstruction by tumor or laryngeal edema can occasionally be confused with asthma. Asthma-like symptoms have been described in patients with glottic dysfunction, endobronchial disease as foreign body, heart (left ventricular) failure, carcinoid tumor, and chronic bronchitis ....etc. In chronic bronchitis there are no true symptom-free periods, and one can usually obtain a history of chronic cough and sputum production as a background on which acute attacks of wheezing are superimposed.

Annex I- McFadden Jr. E. R. Asthma. Section 2; Diseases of the Respiratory System: pages 1508-1512. HARRISON'S PRINCIPLES OF INTERNAL MEDICINE. 16th Edition (2005): 1508-1516. Mc Graw-Hill.

Detailed Appeal / Second

Claim 25 in relation to "A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of allergy"

Claim 25 reads as "A pharmaceutical composition consisting essentially of glycophosphopeptical for oral administration for the treatment of allergy and asthma ....etc", I am arguing that the pharmaceutical composition for the treatment of "allergy" as a group of diseases referred to separately in the patent application, under description of the invention, with enabelment and previous clarification in my reply to the Office Action filed on Aug 2004 with X-ray films clarifying its unique outcome of early clinical testing , and will be detailed later, have been forgotten and overlooked. May I kindly request the allowance of this claimed invention.

Please find my Reply filed Aug 2004, particularly page 2B last paragraph "Current allergic rhinitis medications till page 6B.

I will also include in the mail copy of this Response and Appeal additional updated references related to the same subject.

Other points raised by the Examiner in this Office Action

Election/Restriction

1. Applicant's election with traverse Group I in the reply filed on Aug 4, 2004 is acknowledged.

2. Claims 28-34 are withdrawn as being withdrawn to a non elected invention:

Reply: Agree

Information Disclosure Statement

3. The listing or citing of references in applicant's response is not a proper information disclosure statement.

Applicant's Response Dated April 12, 2006

4. Claims 25-34 are pending. Claims 28-34 are withdrawn from further consideration as being drawn on nonelected invention. An action on the merit of claims 25-27 is considered herein below.

5. The rejection of claims 25-27 under 35 U. S. C. 102(b) as being anticipated by Sanchez Palacios A. et. Al. is maintained for the reasons of record as set forth in the Office Action dated November 21, 2005.

Thank you for your consideration
The Inventor
Nida Nassief

END OF REPORT

X ray follows

ALLERGOL. ET IMMUNOPATHOL., 20. 1 (35-39), 1992

Valoración clínica inmunológica de un modificador de la respuesta biológica, AM3, en el tratamiento de la patología respiratoria infecciosa infantil

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SUMMARY

To assess the immunoclinical effectiveness of a biological response immunomodulator, we used AM3 (glycophosphopeptidic), a glucomannan polysaccharide extracted from the cell wall of a strain of *Candida utilis*, in 20 children with asthmatic bronchitis. They received 2 envelopes (1 g) daily for 4 months.

The results were compared with a control group of 20 untreated children with the same pathology. The following clinical and immunological parameters were assessed in all of them: cough, dyspnea, expectoration, frequency and intensity of the bronchospasm, time of administration of the symptomatic medication, and the delayed cutaneous cells response by means of the intradermoreaction of 5 antigens (*Trichophytom, Candida albicans*, tuberculin, *E. coli* and bacterial antigens).

In the treated group, the immunoferon (AM3) reduced the symptoms, the intensity and frequency of the bronchospasm, and the symptomatic medication (table I, II and III).

In basal conditions, the 40 children presented a state of 75% anergy; after 4 months of treatment, the treated group experienced a 45% decrease in their anergic situation, variation which was statistically significant when compared with the control group.

In our 20 treated patients, AM3 behaved like and immunostimulant, improving the clinical situation and progress in patients with infectious respiratory disorders. We consider that the immunoferon constitutes a coadjuvant therapy to bacterial immunotherapy.

Key words: AM3. Biological response in children. Immunoferon in bronchial asthma.

Allergol. et Immunopathol., 20, 1 (35-39), 1992.

INTRODUCCION

La presencia de un agente extraño en un organismo vivo superior va a dar lugar a una respuesta biológica frente a la agresión, cuyo fin inmediato es la eliminación del mismo.

Esta respuesta defensiva global suele comenzar con la puesta en marcha de mecanismos inespecíficos que forman parte de la respuesta inflamatoria y natural, ya que las dos se interrelacionan frecuentemente para concluir a menudo con una respuesta específica (inmune). Los aspectos cualitativos y cuantitativos de esta respuesta biológica dependen no solamente de la capacidad intrínseca del huésped para responder, sino también de aspectos del agente agresor.

Los modificadores de la respuesta biológica (MRB) han sido definidos como aquellos agentes capaces de modificar las relaciones entre un tumor y su huésped, mediante la manipulación de la respuesta biológica de éste hacia las células tumorales, consiguiendo resultados terapéuticos efectivos (1).

En patología oncológica e infecciosa los MRB pueden realizar sus acciones activando, amplificando o restaurando la reactividad de los mecanismos efectores defensivos o bien inhibiendo a los mecanismos supresores que intervienen negativamente, con la resistencia efectora defensiva del huésped.

El AM3 glicofosfopeptical es un polisacárido glucomanano extraído de la pared celular de una cepa de *Candida utilis* por un proceso fermentativo y absorbido en una matriz inorgánica de fosfato y sulfato cálcicos.

Inmunoferón es un modificador de la respuesta biológica con actividad restauradora inmunohematopoyética sobre los sistemas de inmunidad natural en huéspedes mielo y/o inmunocomprometidos.

En ensayos de farmacología experimental inmunoferón ha demostrado recuperar la actividad citotóxica y antitumoral de células NK y macrófagos en animales inmunodeprimidos por ciclofosfamida y en animales de baja respuesta natural (15). Movilizan células efectoras de la respuesta natural inespecífica a focos inflamatorios no inmunogénicas (4). Incrementa la actividad fagocitaria y bactericida de macrófagos peritoneales y esplénicos frente a diversos agentes patógenos (5).

Bajo el punto de vista de la patología infecciosa, el interferón aumenta la resistencia frente a la infección

provocada por diferentes patógenos, incrementando la actividad bactericida y fagocitaria del macrófago (6, 7), mejora la respuesta terapéutica a los antibióticos en modelos experimentales de infección provocada (13).

El AM3 en tuberculosis pulmonar ha mejorado clínicamente bajo el punto de vista bacteriológico y radiológico en 40 pacientes hospitalizados (3). En broncopatías crónicas e infecciones ORL ha mejorado la evolución clínica reduciendo las recidivas (9, 12). En las profilaxis de infecciones respiratorias recidivantes, asociado a vacunoterapia, redujo significativamente la incidencia y duración de las crisis, la necesidad de instaurar antibióticos y el absentismo laboral por enfermedad (1).

El motivo del presente trabajo ha sido valorar la respuesta clínica inmunológica con AM3 en niños con patología infecciosa respiratoria.

MATERIAL Y METODOS

Pacientes. Se seleccionaron 40 niños no atópicos con clínica respiratoria infecciosa de bronquitis espástica y/o asmática con pruebas cutáneas a neumoalergenos negativas e IgE total normal.

Se dividieron en 2 grupos para el estudio doble ciego de 20 cada uno. Grupo AM3, activo con tratamiento sintomático más inmunoferón sin inmunoterapia bacteriana. Grupo B, control sin inmunoferón con tratamiento sintomático.

Posología. Al grupo A se le administró 2 sobres diarios durante 4 meses (1 gramo diario de glicofosfopeptical).

Valoración clínica. Todos los pacientes acudían a nuestra unidad en régimen ambulatorio. En la primera visita, y a los 4 meses, se valoraban los siguientes parámetros clínicos: tratamiento sintomático (antibióticos, broncodilatadores, antitusivos y mucolíticos) y síntomas (tos, expectoración y disnea), valorándose de 1 a 4 según criterio: 1, ausente; 2, leve; 3, severo, y 4, muy severo. Broncospasmo: en frecuencia en 4 meses e intensidad según criterio: 1, ninguno; 2, reversible con medicación oral; 3, reversible con esteroides inyectables, y 4, reversible con ingreso en urgencias. Efectos secundarios.

Valoración inmunológica. Mediante pruebas intradérmicas para valorar la respuesta cutánea celular siguiendo el método clásico con una solución estéril de 0,1 ml de cada panel de 5 antígenos con control salino negativo. Los antígenos y diluciones recomendadas estándar han sido las de DTH (14). Incluyen los siguientes antígenos: Trichophyton (Dermatophytin «0»1000 PNU/ml, Hollisteer-Stier, USA); Tuberculin (PPD RT23, 5TU/0,1 ml Cheminova, Espanic); *E. coli,* 1.106/ml, Abelló and antígeno bacteriano (vacuna bacteriana 750.106/ml Abelló).

La respuesta cutánea celular se valoraba a las 48 horas midiendo la induración (media de los valores de los diámetros perpendiculares).

Una reacción se consideraba positiva cuando la media de los diámetros fue de = 4 mm. A partir de éstos se establece el «score», suma de los diámetros medios de todas las reacciones positivas. Usando estos scores los 40 pacientes se clasificaron en 3 grupos: 1, anérgicos cuando los scores eran menor de 11 mm; 2, hipoérgicos cuando eran menor de 16 mm, y 3, normales cuando la respuesta era mayor o igual a 16 mm.

Tabla I

Media de la intensidad de los síntomas tos, disnea y expectoración en condiciones basales y a los 4 meses

	Basal			4 meses		
	Tos	Disnea	Expect.	Tos	Disnea	Expect.
GM3	3,1	3,8	2,9	1,9	2,1	1
GB.....	2,9	3,9	3,8	2,8	3	3,5

RESULTADOS

La edad media de nuestros pacientes fue de 3 años y 7 meses, con un rango de 2-7 años. El tiempo de evolución de la enfermedad fue de 2 años y 4 meses y la distribución por sexos 11 niñas y 29 varones (36/72%). Por grupos AM3 (edad, 3,6; rango, 2-7; sexo, 6 h/14 v). GB (edad, 3,9; rango, 2-6; sexo, 5 h/15 v).

En la Tabla I vienen expresados la media de la intensidad de los síntomas tos, expectoración y disnea; en los grupos GM3 y GB en condiciones basales y a los 4 meses. Como puede observarse, la intensidad de los 3 síntomas es menor a los 4 meses en el grupo tratado con inmunoferón.

En la Tabla II vienen expresadas la frecuencia e intensidad del broncospasmo como la media del número de crisis de todos los pacientes en condiciones basales y a los 4 meses. El grupo GM3 presentó una disminución en intensidad y frecuencia de las crisis con respecto al B, con una $p < 0,001$.

En la Tabla III vienen reflejados el número de días con tratamiento antibióticos, mucolíticos y antitusígenos a lo largo de los 4 meses y la media del número de días por pacientes. En el grupo AM3 el consumo de antibióticos mucolíticos y antitusígeno fue menor que el B, con una $p < 0,001$.

Tabla II

Media de la frecuencia e intensidad del broncospasmo

Grupos	Basal		4 meses		
	$\bar{x}$	$\bar{x}$	$\bar{x}$	$\bar{x}$	
	N.° broncospasmo	Intens.	N.° broncospasmo	Intens.	
AM3	12	3,1	6,2	2,1	p-0,001
B	11,8	3,4	12	3	p NS

Tabla III

Tiempo de administración de la medicación sintomática

Tratamiento	AM3		Basal	
	Total días	Días/ paciente x ± DS	Total días	Días/ paciente x ± DS
Antibióticos	110	8 ± 2,1	220	21 ± 2,07
Mucolíticos	530	10 ± 2,5	890	40 ± 3,8
Antitusígenos .	80	740 ± 2,5	200	13 ± 3,2

En el grupo de los 20 pacientes tratados con inmunoferón AM3 no observamos efectos secundarios con la medicación a lo largo de los 4 meses. En las Tablas IV y V se expresan los resultados de la respuesta cutánea celular y se pueden observar cómo en condiciones basales ambos grupos AM3 y B tienen una respuesta celular similar, presentando anergia, es decir, una suma de scores menor de 11 mm en 16 pacientes y 14 para ambos grupos, lo que representa el 75% del total. En un estadio hipoérgico aparecen el 25% restante, no existiendo una respuesta cutánea celular en ninguno de ellos.

Tras el tratamiento con inmunoferón en el grupo AM3, al cabo de 3 meses se modifica la respuesta cutánea celular, disminuyendo en 9 pacientes el estadio de anergia del 80 al 35%. Este hecho no ocurre con el grupo control B, en el cual la respuesta no se modifica con una p no significativa.

En la Figura 1 se expresan estos resultados en el eje de abscisas, se expresan el número de pacientes y en la ordenada los milímetros de la respuesta cutánea para ambos grupos; en condiciones basales los 2 grupos son homogéneos y a los 3 meses las 2 líneas en sus valores se cruzan, expresando la disminución de la anergia en el grupo tratado con inmunoferón, siendo la diferencia significativa con respecto a su situación basal $p < 0,33$.

Ninguno de los 20 pacientes tratados con 1 gramo de glicofosfopeptical durante los 4 meses tuvieron efectos secundarios y las cifras de calcemia no se modificaron.

DISCUSION

La tórpida evolución que presentan muchos niños afectos de patología infecciosa respiratoria, sean o no atópicos, la achacamos a una falta de inmunoestimulación específica o inespecífica y del tratamiento sintomático, fundamentalmente la ausencia de antibioterapia.

La existencia de un foco infeccioso rinosinusal en el curso de un asma bronquial y/o bronquitis asmática empeora, desencadena o cronifica dicho proceso, de ahí la importancia de utilizar los antibióticos idóneos y la inmunoestimulación (10, 11, 14).

El AM3 (glicofosfopeptical) se comporta como un inmunomodulador de la respuesta biológica según trabajos publicados sobre farmacología experimental y clínica.

Muchos niños tienen alteraciones cualitativas y/o cuantitativas de la inmunidad humoral y celular en la respuesta biológica defensiva global. La disminución del sistema IgA secretor en el árbol respiratorio explicaría las reagudizaciones infecciosas respiratorias.

En nuestros 20 niños tratados se demuestra una mejoría clínica e inmunológica tras los 3 meses con el AM3. La intensidad de los síntomas tos, expectoración y disnea disminuye, así como la frecuencia e intensidad del broncospasmo. La medicación sintomática, sobre todo antibióticos y antitusígenos, fue mejor que en el grupo control.

Para la valoración de la respuesta de hipersensibilidad cutánea retardada hemos utilizado la técnica clásica de intradermorreacciones a una batería de 5 antígenos; esta fue empleada siguiendo las recomendaciones vigentes para su aplicación e interpretación de forma estandarizada (2). Desechamos el Multitest CMI (Institute Merienx, Lyon, Francia), porque trabajos previos demuestran que la técnica clásica de intradermorreacciones tiene una sensibilidad superior al Multitest en la detección de anergia en la población

Tabla IV

Respuesta cutánea celular (RCC) en condiciones basales

RCC	AM3 N.° de pacientes	GB N.° de pacientes
Anergia < 11 mm	16 (80%)	14 (70%)
Hipoérgicos < 16 mm ...	4 (20%)	6 (30%)
Normales ≥ 16 mm	0	0

Tabla V

Respuesta celular cutánea a los 4 meses de tratamiento

Respuestas	AM3	GB
Anergia < 11 mm	7 (35%)	16 (80%)
Hipoérgicas < 16 mm.....	13 (65%)	4 (20%)
Normales ≥ 16 mm	0	0

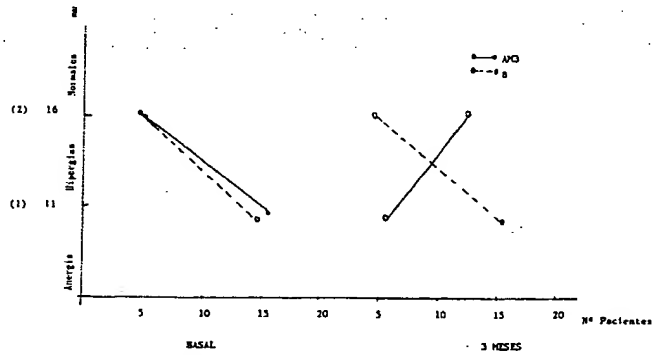

Fig. 1.—Evolución de la respuesta cutánea celular en 3 meses con y sin AM3.

inmunodeprimida y además reúne todos los criterios que requiere una batería de antígenos para medir la respuesta de hipersensibilidad cutánea retardada (6).

RESUMEN

El AM3 (glicofosfopeptical) es un polisacárido glucomanano extraído de la pared celular de una cepa de *Candida utilis*. Los ensayos de farmacología experimental y clínica han demostrado que el inmunoferón recupera la actividad citotóxica antitumoral, de células NK y macrófagos, moviliza células efectoras de la respuesta natural inespecífica a focos inflamatorios no inmunogénicos, incrementa la actividad bactericida, favorece la respuesta proliferativa T en algunas formas de inmunocompromiso inducido por citostáticos. Bajo el punto de vista clínico en tuberculosis pulmonar, broncopatías crónicas infecciosas ORL, herpes simple cutaneomucoso y en la profilaxis de infecciones respiratorias ha mejorado la evolución clínica en intensidad y recidivas, con reducción del tratamiento sintomático.

El propósito de nuestro trabajo ha sido valorar la eficacia clínica inmunológica de 20 pacientes afectos de bronquitis asmática. Recibieron 1 gramo diario de glicofosfopeptical durante 4 meses. Los resultados se compararon con un grupo control de 20 niños no tratados con AM3 con la misma patología. En todos ellos se valoraron los siguientes parámetros clínicos e inmunológicos: tos, disnea, expectoración, frecuencia e intensidad del broncospasmo, tiempo de administración de la medicación sintomática y la respuesta cutáneo celular retardada mediante la intradermorreacción de 5 antígenos *(Trichophyton, Candida albicans, Tuberculin, E. coli* y antígenos bacterianos).

El inmunoferón (AM3) consigue en el grupo tratado una disminución de los síntomas, de la intensidad y frecuencia del broncospasmo y de la medicación sintomática. Modificó la respuesta cutánea celular en 9 pacientes que se encontraban en una situación de anergia, lo que representa un cambio inmunológico celular en un 45% de los pacientes tratados. Este hecho no ocurre con el grupo control, siendo la diferencia estadísticamente significativa. Pensamos que el AM3 constituye un tratamiento coadyuvante de la inmunoterapia bacteriana.

La tórpida evolución presentada por algunos niños con patología respiratoria infecciosa con o sin atopia la achacamos a la falta de una inmunoestimulación.

Con el propósito de valorar la eficacia clínica inmunológica de un inmunomodulador de la respuesta biológica utilizamos el AM3 (glicofosfopeptical), que es un polisacárido glucomanano extraído de la pared celular de una cepa de *Candida utilis* en 20 niños afectos de bronquitis asmática. Recibimos 2 sobres diarios (1 g) durante 4 meses.

Los resultados se compararon con un grupo control de 20 niños con la misma patología no tratados. En todos ellos se valoraron los siguientes parámetros clínicos e inmunológicos: tos, disnea, expectoración, frecuencia e intensidad del broncospasmo, tiempo de administración de la medicación sintomática y la respuesta cutáneo celular retardada mediante la intradermorreacción de 5 antígenos *(Trichophyton, Candida albicans, Tuberculin, E. coli* y antígenos bacterimus).

El inmunoferón (AM3) consigue en el grupo tratado una disminución de los síntomas, de la intensidad y frecuencia del broncospasmo y de la medicación sintomática (Tablas I, II y III).

En condiciones basales los 40 niños presentaron un estado de anergia en el 75%; a los 4 meses de tratamiento el grupo tratado experimentó una disminución de la situación de anergia en un 45%, siendo esta variación estadísticamente significativa con respecto al grupo control.

En nuestros 20 pacientes tratados el AM3 se comporta como un inmunoestimulante, mejorando la situación y evolución clínica en pacientes con patología respiratoria infecciosa. Pensamos que el inmunoferón constituye un tratamiento coadyuvante de la inmunoterapia bacteriana.

Palabras clave: AM3. Respuesta biológica en niños. Inmunoferón en bronquitis asmática.

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2. Patent a[...] **9904777.1** -2 MAR 1999
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Patents ADP number *(if you know it)* Tel: +974 650 664 Fax: +974 650 073

If the applicant is a corporate body, give the country/state of its incorporation 761307800[1]

4. Title of the invention NOVEL METHOD
AN ASTHMA THERAPY THAT ACT ON EOSINOPHILS AND/or T-Lymphocytes

5. Name of your agent *(if you have one)* Correspondance To 7613086001

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/		

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/	

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a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d)) /

THE DRUG TO BE PATENTED:

(1) **COMMERCIAL NAME:** INMUNOFERON

mentioned in the text as drug (N)

ACTIVE INGREDIENTS: GLICOFOSFOPEPTICAL (3905-W)
(Fosfoglicopeptical)

sour. MARTINDALE THE EXTRA PHARMA

PRODUCING COMPANY:
LABORATORIOS ANDROMACO S.A.,
AZCONA, 31 - 28028, MADRID, SPAIN.

THE NEW TRADE NAME:

/

A NEW DRUG APPPLICATION (NDA) FOR THE ABOVE MENTIONED DRUG, IN THE TREATMENT OF DISEASES MEDIATED BY TYPE 1 HYPER SENSITIVITY REACTION, MAINLY ASTHMA

(2) A NOVEL B.R.M. (BIOLOGIC RESPONSE MODIFIER)
NIGELLA SATIVUM NATURAL HERB
ORALLY ADMINSTERED
PURE POWDER IN CPSULES OR SUSPENSIO
200 - 800 mg / dose
THREE TIMES / day
INDICATIONS : PAGE 2

(3) OTHER BIOLOGIC RESPONSE MODIFIERS (IMMUNOMODULATORS) IN TYPE I HYPERSENSITIVITY REACTON ON SHORT TERM BASIS

Indications for the therapeutic use of Substance (A):

NIGELLA SATIVUM NATURAL HERB

For the treatment of diseases associated with a defect in cell mediated immunity

I – Diseases caused by type I hyper sensitivity, such as:

1. Asthma.
2. Laryngeal oedema (Angioneurotic).
3. Allergic Rhinitis.
4. Allergic Conjunctivitis.
5. A topic dermatitis.

II- Acute & recurrent UTI.

III- Pelvic inflammatory diseases.

IV- Viral respiratory tract infections (flue & influenza).

V- Other viral infections.

VI- Cancer therapy.

2A 42B FOLLOWS ON LINE ARTICLES AT THE END

Advantages:

The following advantages had been noticed, with drug (N) and substance (A) therapy, for **ASTHMA** during clinical trial:

I- **Enables the patient to live a more rather normal life** as it is capable of.
Meeting most of the goals of asthma therapy mentioned earlier.

II- **Short course** of therapy of **30 capsules,** or what is equivalent in-total during the course.

III- The course of treatment can be repeated as needed at intervals.

IV- **Enabling the patient to reduce** other conventional anti-asthma therapy because they don't need them as they are symptom-free.

V- **Tapering corticosteroids** and weaning from it.

VI- **Patient compliance is very good,** because this treatment is capable of achieving the confidence of the chronically ill patient with some psychological element from the disease. ie, suffering continuous use of conventional therapy with partial relief of his symptoms.

VII- **Side effects are few,** mild epigastric pain some times occurs with a large dose,
No other side effects were noticed during treatment.
Complete blood picture shows **no** abnormalities.
Blood urea and creatinin were normal during treatment.
Liver function (total serum bilirubin, SGPT, alkaline.
Phosphatase) were normal.
General urine analysis is normal.

Substance (A) had been used in humans for quite some time with out a noticeable toxic or undesirable effects.

Drug (N) is already present in the market for human use.

VIII- The results of this therapeutic approach lead to the formation of theory in the immunopathogeneses of type I hyper sensitivity namely **a selective switching-off of the eosinophilic inflamtion by stimulating the function of regulatory T Lymphocytes**

ASTHMA

Definition:

Asthma is a chronic inflammatory disorder of the airways. This causes recurrent episodes of wheezing, breathlessness, chest tightness and cough particularly at night and /or in the early morning. These symptoms are usually associated with wide spread but variable airflow limitation, and is at least partly reversible either spontaneously or with treatment.[1]

Incidence

There are between 100 and 150 million people in the world, including many children, who do not take breathing for granted. For them it can be a life and death struggle against recurrent attacks of breathlessness and wheezing caused by asthma. Each year, arround 180 000 of these sufferers lose the battle and die of the disease.[2]

PATHOGENESIS OF ASTHMA

An understanding of the pathogenesis and pathophysiology of this disorder provides the opportunity **for improved therapeutic intervention.**[3]

The pathogenesis of asthma is **complex and not fully understood**, it involves a number of cells, mediators, nerves and vascular leakage that can be activated by several different mechanisms, of which exposure to allergens is the most important.[4] (Fig. 1)

Numerous hypotheses have been put forth to explain the clinical syndrome of asthma. Recently, an appreciation of airway inflammation in asthma has led to reevaluation of all the concepts in the pathogenesis. Various in vivo and in vitro experiments suggest that, in susceptible host, **a T-cell mediated immune response to inhaled antigen occur.**[3]

The pathological features of asthma have also been investigated in patients by means of sputum examinations, bronchoatveolar Lavage (BAL) and endobronchial biopsy, all of inflamatory cells. Eosinophils in the sputum have been suggested as a marker of asthma.[3]

In patients dying of fatal exacerbation of asthma, microscopical examinations of the lungs revealk many changes, the most readily apparent and consistent features of the inflammatory cell infiltrate, particularly eosinophils, Lymphocytes and other inflammatory cells.

Endobronchial biopsy specimens reveal inflammatory cell influx similar to that found in postmortem speciems of airways, **even when diseases are relatively quiscent.**[3]

Immediate hypersensitivity reaction refers to a collection of signs and symptoms comprising respiratory, cutaneous, cardiovascular, gastrointestinal and systemic responses to a variety of pharmacologically active proinflammatory substances called **mediators.**[3b]

These reactions require the concerted interactions of sensitizing antibodies, specific target cells and mediators.[3b] **Any one of these can be targeted as a therapeutic weapon.**

Antibodies responsible for immediate hypersensitivity reaction. First where described by Prausnitz and Kustner in 1921, 40 years later it was purified and called **IgE**. It is a cytophilic antibody that binds specific surface receptors of mast cells. Cross- linking of two IgE antibody molecules results in mast cell degranulation. IgE is under complex regulatory control, genetic influences are important.[3]

In humans type 1 reaction is mediated by IgE antibodies. **The differentiation of IgE secreting B cells is highly dependant on the induction of CD4+ helper T cells of TH2 type.** The first step in the synthesis of IgE is the presentation of the antigen (allergene) to precursors of TH2 cells by antigen presenting **dendritic cells**. The newly minted TH2 cell produce a cluster of **cytokines**, including IL-3, IL-4, IL-5 and GM-CSF, of these, IL-4 is absolutely essential for turning on the IgE-producing B cell.[5]

Humans T helper (CD4+) cells can be divided into subsets, TH1 and TH2, based on the profile of cytokines they produce. This is important in selecting effector function.(Fig.2)

TH1 cell: involved in cytotoxic, inflammatory and delayed type hypersensitivity. Interferon-gamma (IFN) has modulatory effect on immune function important in transfer of antigenic information to T lymphcytes.[7]
Reduced IFN at birth is noticined in babies born to families with a history of atopic allergic diseases.[7]

TH2 cell: encourages production of IgE & regulation of allergic response. IL 5 released by TH2 cells are chemotactic for eosinophils & enhance their release of mediators & cytokine.[6]

Cytokines from TH1 cells inhibit the action of TH2 cells & vice versa.
The cytokine environment determines which T-cell subset is produced from TH0 cells, and therefore which pathway B cells will take. (Fig.3)

T cells deficiency is associated with atopy: there is substantial evidence for a role for T-cells in both the development & suppression of IgE responses.
This led to the earlier suggestions that a **defect in T-cells, and in particular suppressor T-cells could be involved in the etiology of atopy** [6].

Human mast cells have been demonstrated to synthesize and release a variety of cytokines and growth factors, postulated in eosinophil growth, maintenance, recruitment, and activation; in IgE synthesis; and in fibrosis.

The pattern of mast cell cytokine synthesis is similar to TH2 helper T Lymphocytes and may be important contributor to allergic inflammation, such as that noted in asthma. *

Eosinophiles: these are non- dividing granular cells which arise principally in the bone marrow. Eosinophil differentiation, like that of all leukocytes is influenced by cytokines[7], they constitute up to 5% of white blood cells in healthy individuals and appear to be used selectively for fighting parasitic infection, they participate in hypersensitivity (allergic) reactions [4]. In allergic inflammatory disorders chaemokines such as Macrophage chaemoattractant protein (MCP) and eotaxin from macrophages, activated leukocytes & endothelial cells bind to receptors in the eosinophiles, inducing cell migration and activation [4].

Extravasation of leucocytes involve :

1. In the lumen arigration , rolling, adhesion.
2. Transmigration across the endothelium (diapedesis)
3. Migration in interstitial tissues toward a chemotactic stimulus.(robbins P 56:

Eosinophil Chaemotactic Factors:

Various factors are released from different cells that has the ability to attract eosinophils to migrate to the tissue, these include:

- Mast cell degranulation & basophils release an Eosinophil Chemotactic Factor (ECF) , **A low – molecular weight peptide (300-500 daltons)** . Also a preformed , immunologically releaseable , **chemotactic factor (1500-3000 daltons)** whith specificity for eosinophils .[7]

- Similar factors have been identified in the circulation of patients after **experimental induction of physical urticaria** or antigen provoked bronchospasm.

- Other ECF can be generated by **the lipoxygenase – dependant pathway of arachidonic acid metabolism .**[7]
- **Platlet Activating Factor (PAF)** is also a potent leukoattractant, particularly for eosinophils.

- **Rantens**

MANAGEMENT OF ASTHMA

The principles of management of asthma are based closely on the guidelines for the management of asthma produced by the British Thoracic society and also the International Consensus Report on the diagnosis and management of asthma (Fig.)

Expert panel report 2 from the National Asthma Education and prevention program of the National Heart, Lung and Blood Institute recommended a step wise approach to therapy (tab) (center 99).

Management consists of:

A) Environmental control and antigen avoidance.

B) Immunotherapy or hyposensitization.

C) Pharmacological agents, that can be divided into:

1)**Quick relief mediation**: taken to promote prompt reversal of acute airway obstruction and relief of accompanying symptoms by direct relaxation of smooth muscle, table (ref. current).

2)**Long term control** of persistent asthma, also referred to as maintenance, controller or preventive medications act primarily to attenuate airway inflammation. table (ref. current 99).

Table 9–5. Selected drugs for obstructive airway diseases.[1]

Drug	Important Formulations	Usual Adult Dosage (Stable Patient)	Comments
BRONCHODILATORS			
Sympathomimetics			
Albuterol (Proventil, Ventolin, Volmax)[2]	Metered-dose inhaler (90 µg/puff; 200 puffs/inhaler)	1–4 puffs every 4–6 hours[3]	Preferred formulation in most cases. Clinically similar to metaproterenol but slightly longer duration of action.
	Nebulized solution (0.5%)	0.5 mL plus 2.5 mL normal saline every 4–6 hours[3]	Administer with powered nebulizer or, rarely, by IPPB.
	Unit dose solution (0.083%)	One 3 mL dose every 4–6 hours[3]	Administer with powered nebulizer.
	Powder (Ventolin Rotocaps) (200 µg)	One 200 µg capsule every 4–6 hours	Requires Rotohaler to inhale.
	Tablets (2 mg, 4 mg) Syrup (2 mg/5 mL)	2–4 mg orally every 6–8 hours	An extended-release 4 mg tablet is available for use every 12 hours (Proventil Repetab). Volmax extended-release tablets are available in 4 mg and 8 mg strengths.
Salmeterol (Serevent)	Metered-dose inhaler (21 µg/puff; 120 puffs/inhaler)	2 puffs every 12 hours	Long-acting agent for maintenance therapy of asthma. Should not be used for acute relief of symptoms. The most expensive β_2 agonist.
Metaproterenol (Alupent, Metaprel)	Metered-dose inhaler (650 µg/puff; 200 puffs/inhaler)	1–4 puffs every 3–4 hours (or more frequently)[3]	Preferred formulation in most cases.
	Nebulized solution (5%)	0.3 mL plus 2.5 mL normal saline every 3–4 hours[3]	Administer with powered nebulizer or rarely, by IPPB. Also available as single-dose vial.
	Unit dose solution (0.4% and 0.6%)	One 2.5 mL dose every 4–6 hours[3]	Administer with powered nebulizer.
	Syrup (10 mg/5 mL)	2 tsp orally every 6–8 hours	Tremor, nervousness, palpitations common. Oral formulation therefore not recommended.
	Tablets (10 mg, 20 mg)	20 mg orally every 6–8 hours	
Bitolterol (Tornalate)[2]	Metered-dose inhaler (370 µg/puff; 300 puffs/inhaler)	2–3 puffs every 6–8 hours[3]	
	Inhalation solution (0.2%)	1.25 mL every 6–8 hours[3]	
Pirbuterol (Maxair)[2]	Metered-dose inhaler (200 µg/puff; 300 puffs/inhaler)	2 puffs every 4–6 hours[3]	
	Breath-activated metered-dose inhaler (200 µg/puff; 400 puffs/inhaler)	2 puffs every 4–6 hours[3]	
Terbutaline[2] (Brethaire)	Metered-dose inhaler (200 µg/puff; 300 puffs/inhaler)	2–3 puffs every 4–6 hours[3]	
(Brethine, Bricanyl)	Tablets (2.5 mg, 5 mg)	2.5–5 mg orally 3 times daily	Tremor, nervousness, palpitations common. Oral formulation therefore not recommended.
	Subcutaneous injection (1 mg/mL)	0.25 mg subcutaneously; may be repeated once in 30 minutes	Slow onset of action (30 minutes). Not limited to β_2-adrenergic stimulation.
Isoetharine (Bronkometer, Bronkosol)	Metered-dose inhaler (340 µg/puff; 200 puffs/10 mL inhaler)	1–4 puffs every 3–4 hours[3]	

SO: CURRENT MEDICAL DIAGNOSIS AND TREATMENT
1997 AU: TIERNEY ETAL

Table 9–5. Selected drugs for obstructive airway diseases.[1] (continued)

Drug	Important Formulations	Usual Adult Dosage (Stable Patient)	Comments
Isoetharine (cont'd)	Nebulized solution (1%)	0.5 mL of 1% solution plus 1.5 mL normal saline every 2–4 hours	Adminsiter with powered nebulizer or, rarely, by IPPB.
Isproterenol (Isuprel, others)	Metered-dose inhaler (131 µg/puff; 200 puffs/10 mL)	1–3 puffs every 2–4 hours	
	Nebulized solution (0.5%; 1% also available)	0.5 mL of 0.5% solution plus 1.5 mL normal saline every 2–4 hours	Administer with powered nebulizer or, rarely, by IPPB
Epinephrine (many brands)	Metered-dose inhaler (200 µg/puff)	1 or 2 puffs every 2–4 hours	Available without prescription. β_1 and α stimulation limit usefulness.
	Subcutaneous injection (0.1%; 1:1000)	0.3–0.5 mL subcutaneously; may be repeated once in 30 minutes	Use with caution in older patients or those with tachycardia, hypertension, or arrhythmia. No more effective than inhaled β_2 agonist.
Anticholinergics Ipratropium bromide (Atrovent)	Metered-dose inhaler (18 µg/puff; 200 puffs/inhaler)	2–4 puffs every 6 hours	More potent than sympathomimetics in COPD. Minimal side effects.
	Unit dose inhalation solution (0.02%)	One 2.5 mL dose every 6–8 hours	
Theophyllines Theophylline, oral (many brands)	Sustained-release tablets and bead-filled capsules	200 mg orally every 12 hours initially; thereafter, 200–600 mg orally every 8–12 hours	Maintenance dose is guided by serum theophylline level. Therapeutic level is 10–20 µg/mL. Absorption varies with brand. Formulations are also available for administration every 24 hours.
Aminophylline	Intravenous	Loading dose is 5.6 mg/kg over 30 minutes for a person not using oral theophylline; maintenance dose is 0.7 mg/kg/h by constant infusion pump—lower if patient has liver disease or heart failure or is receiving erythromycin or cimetidine.	Seldom indicated. Calculate dose from lean body mass. Monitor serum theophylline level.
CORTICOSTEROIDS Beclomethasone dipropionate (Beclovent, Vanceril)	Metered-dose inhaler (42 µg/puff; 200 puffs/inhaler)	2 puffs 4 times daily, or 4 puffs twice daily	Rinse mouth with water after use to prevent oral candidiasis; use 30 seconds after inhaled sympathomimetic to control cough and airway irritation. Spacer devices also helpful to prevent oral candidiasis.
Triamcinolone acetonide (Azmacort)	Metered-dose inhaler with spacer (100 µg/puff; 240 puffs/inhaler)	2 puffs 4 times daily, or 4 puffs twice daily	Cough and wheezing after inhalation are reported to be less than after inhalation of beclomethasone.
Flunisolide (AeroBid)	Metered-dose inhaler (250 µg/puff; 100 puffs/inhaler)	2–4 puffs twice daily	Dosing frequency of twice daily offers an advantage.
Prednisone (several brands)	Tablets (2.5, 5, 10, 20, and 50 mg)	Acute bronchospasm: 40–60 mg (1 mg/kg) every 24 hours Chronic bronchospasm: 5–40 mg daily or every other day	Discontinue after 14 days if possible.
Methylprednisolone sodium succinate (several brands)	Intravenous injection (vials of 40, 125, 500, 1000, and 2000 mg)	0.5–1 mg/kg every 6 hours	Clinical response may be delayed for several hours.

(continued)

Table 9–5. Selected drugs for obstructive airway diseases.[1] (continued)

Drug	Important Formulations	Usual Adult Dosage (Stable Patient)	Comments
Hydrocortisone sodium succinate (several brands)	Intravenous injection (100, 250, 500, and 1000 mg)	4 mg/kg every 6 hours	Clinical response may be delayed for several hours.
ANTIMEDIATORS			
Cromolyn sodium (Intal)	Metered-dose inhaler (800 µg/puff; 200 puffs/14.2 g canister)	2–4 puffs 4 times daily	Clinical response may require 2–4 weeks of treatment. Useful only for prophylaxis; younger patients with asthma are more likely to benefit. To prevent bronchospasm, cromolyn may be used 15–30 minutes before exercise or exposure to cold air or allergens.
	Nebulized solution (20 mg/2 mL ampule)	20 mg 4 times daily by powered nebulizer	
Nedocromil sodium (Tilade)	Metered-dose inhaler (1.75 mg/puff; 112 puffs/inhaler)	2 puffs 4 times daily	Maintenance therapy for asthma.

[1]Only drugs available in the United States are listed.
[2]Preferential effect is on β_2-adrenergic receptors.
[3]More frequent dosing for acute or severe episodes of bronchoconstriction is acceptable.

All these schedules of therapy are aiming at meeting the **goals of asthma therapy**.

Various authors put various goals. In general all of them are aiming at reduction of the patient sufferings and enable him to live a rather normal life, while using a single drug or a combination of various drugs as needed by the particular patient.

Although individuals may have specific therapeutic goals, **general goals include:**

(1) Optimal control of asthma with the use of the least amount of medications possible and minimal side effects.

(2) Reduction in hospitalization and emergency care visits.

(3) Prevention of nocturnal symptoms

(4) Tolerance to physical activity appropriate for the patients age.

(5) Improvement of pulmonary function.

(6) Minimization of lost time from school, work, or daily activities (Ref.)

In a special issue, Allergy, the Journal of the European Academy of Allergy and Clinical Immunology (EAACI) defined the goals of asthma management as follows:

(Allergy Supplement (1995) 27:50)

1. Enable the patient to enjoy a normal life, comparable to that of a healthy person.
2. Maintain respiratory function as a close as possible to normal levels.
3. Prevent cough and dyspnoea at night ensuring a good sleep.
4. Prevent asthma exacerbation.
5. Minimize side effects from asthma,.
6. Reduce mortality.

Other Criteria to approach long term treatment

The goals of asthma therapy are to minimize chronic symptoms that impairs normal activity (includes exercise), to prevent recurrent exacerbation, to minimize the need for emergency department visits or hospitalizations, and to maintain near-normal pulmonary function. These goals should be met while providing optimal pharmacotherapy with the fewest adverse effects (Ref.)

The drawbacks of this conventional therapy are:

(1) Frequent dosage, several times/day.

(2) Side effects are prominent especially with oral drugs.

(3) This conventional therapy fails to meet the goals mentioned, as evident from the presence of persistent asthma of different grade (mild, moderate, sever) with treatment.

(4) Patient compliance is poor due to the chronicity of the illness and frequent use of medication.

(5) Psychological sufferings.

(6) Frequent absence from school and work.

(7) The economic load on the family.

(8) Disruption of family dynamics.

(9)Asthma must be regarded as a potentially life- threatening disorder even in extremely mild cases. The incidence of mortality is increasing. Keeping in mind the huge member of patients involved world wide, it is clear that the problem need an urgent action.

Humanitarian Efforts are Needed to Help the Asthmatics

The World Health Organization's (WHO) 1997 report indicated an increasing prevalence of asthma in the past two decades in both children and young adults probably resulted from environment pollution. Asthma together with a chronic bronchitis and emphysema kill almost 3 million people globally each year. Asthma can be fatal even in young people. The *reasons are poorly understood.*

Asthma is responsible for at least 2% of health-case costs in affluent population. WHO stresses the necessity for developing strategies aimed at reducing the morbidity and mortality in a cost-effective way.

The National Heart Lung and Blood Institute (U.S.A.) and the WHO have jointly instigated a global initiative on asthma (GINA) to design and deliver an effective asthma management and prevention programmes.

With this intention we are targeting a novel site in the immunolopathologenosis of asthma.

Clinical trials show that this inventive therapy has a long-term effect, therefore it will be useful to throw light on the mode of action of drugs used for long term control of persistent asthma.

Asthma Prophylactics

Mast cell stablizers

are safe drugs

(1)disodium cromoglycate (Intal) spincaps for inhalation nedocromil sodium (Tilade) prevent early and late allergic reactions. They work preventively to inhibit information and antigen increase in airway hyperactivity- maintenance therapy requires 3 or 4 daily doses and it can take as long as 4 weeks for an effect to be appreciated. Therapy should be continuos

(2)ketofiren (zaditen).

Properties/Actions

Is a non-bronchodilator anti-asthmatic drug with marked anti-anaphylactic properties and a specific antihistamine effect.

Laboratory experiments, both in vitro and in vivo, have revealed the following properties of zaditen, which may contribute to its anti-asthmatic activity:

* Inhibition of both the acute bronochoconstrictor response to PAF (Platelet Activating Factor) and of PAF-induced airway hyperrespon-siveness.
* Inhibition of PAF-induced accumulation of eosinophils in the airways.
* Inhibition of the release of such chemical mediators as histamine and leukotrienes.
* Antagonism of acute bronchoconstriction due to leukotrienes.
* Reversal or prevention of experimentally induced isoprenaline tachy-phylaxis.

In addition, zaditen excerpts powerful and sustained H_1 receptor blocking activity which can be clearly distinguished from its anti-anaphylactic properties.

3)Corticosteroids: are extremely potent anti-asthma agents. There are severally topical formulations delivered by metered dose inhalers. Usual maintenance dose is 100-200 microgram twice daily.(conns)

Action of Corticosteroids:

Corticosteroids such as prednisolon interfere at many points in the immune response, i.e. lymphocyte recirculation, inhibit neutrophil adherence to vascular endothelium in

an inflammatory and suppression mononcyte macrophage functions such as microbicidal activity and response to lymphokines. (Davidson chapter immunologic disease).

Four weeks treatment with recommended dose was associated with significant improvement in peak flow, FEV1 & rescue Salbutamol use in asthmatic subjects . but **was not** associated with large reductions in markers of eosinofilic inflamation , broncovascular permiability , or **mucus hypersecretion .**

Effect of low- dose Beclonethosone dipropionate on asthma control and airway inflamation. (Fahy- JV; BOUSHEY- HA
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A Breakthrough in the Treatment of Asthma and Allergy by a Novel Use of an Old Drug Possibly Leading to a New Hypothesis in Their Immunopathogenesis.

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Abstract:

A drug (N), present in the market, indicated for diseases unrelated to type 1 hypersensitivity, was linked with allergy in a novel way, using it in a non-routine indication and dosage. The theory was build up during the last 4 years in 3 stages. Stage I: concieving the idea of the link between (N) and allergy. Stage II: proving its utility and reduction to practice, by a double-blind placebo controlled clinical trial, involving 120 subjects to assess its beneficial therapeutic effect in various acute and chronic allergies, depending on symptom scoring and chronological events. The treated patients showed marvelous symptomatic improvement and possible long-term effect, hence proceeding to stage III in which, a study was designed to objectively evaluate the therapeutic effect of (N). Nine severe chronic asthmatics receiving maximal doses of anti-asthma drugs and corticosteroids were treated with (N) administered orally according to a schedule of 30 capsules (or what is equivalent) in total, over the whole study period, while continuing their previous therapy. Within 3-4 days of starting (N) therapy, there was more than 65% improvement in their global self-assessment scoring, enabling the patient to discontinue anti-asthma drugs and corticosteroids. Pulmonary function tests revealed a clinically significant increase in parameters reflecting obstructive lung disease. Several months of follow up showed that the chronically disabled patients came to lead a rather normal life with minimal drugs used on need only. There was a tremendous decrease in the quantity of sputum and in its easinophil content.

Such result most likely indicates a selective switching-off of the inflammatory process. It is a breakthrough in asthma therapy and might lead to a new approach in the immunopathogenesis of asthma and allergy. The response of both atopic and nonatopic asthmatic patients to this treatment might give an additional evidence of similarity of both types going with Humbert's suggestions.

Introduction:

Type I IgE mediated hypersensitivity of Coombs and Gell, is the basic harmful immunologic mechanism in different types of acute and chronic allergies including asthma[28], an extremely common disorder. Five per cent of the population are currently symptomatic, and 7-10% of children[4,15], with great physical and psychological sufferings, high hospitalization and death rates[5,7,22].

From the immunopathologic point of view, asthma is considered as multifactorial disease. It develops on the basis of genetic predisposition and involves characteristic sequence of changes in immune functions[38]. The histopathology of both atopic and nonatopic asthma is a chronic desquamative eosinophilic bronchitis[5,30,38], IgE dependent release of mast cell mediators is responsible for extrinsic allergic asthma[15]. T-cell derived lymphokines are intimately involved in the regulation of IgE production[1] and are responsible for immediate and late inflammation. A series of cell to cell interactions mediated through various cytokines are producing the pathologic features, of the disease[13,18,23,26,27,31,33]. A balance of functionally distinct macrophages needs to be maintained to regulate T-cell reactivity in the lung, *atopic asthma is promoted by dysregulation of T-cell mediated mechanisms*[41].

Eosinophils are a key inflammatory cells in asthma, it is pathologically considered as a chronic desquamative eosinophilic bronchitis. There is now a very persuasive evidence that eosinophils are important inflammatory cells in bronchial mucosal damage and hyperresponsiveness[1,8,30]. Inflammation leads to airway obstruction, as a result of oedema, broncho-spasm, mucus hypersecretion with changes in the viscoelastic properties[15,25,32].

Humbert, M. provided evidence for *similarities in the immunopathogenesis of both atopic and nonatopic asthma*, which are clinically distinct, both being mediated by IgE associated with eosinophilic inflammation IL-4 and IL-5 are essential mediators in both types[39].

Various results of inflammation or Factors involved in it, can be addressed as a therapeutic goal. The ultimate test for a new drug is therapeutic efficacy in clinical trial[27].

The goals of asthma management are[18].

1. Enable patients to enjoy normal life, comparable to that of healthy persons.
2. maintain respiratory function as close as possible to normal levels.
3. Prevent cough and dyspnoea at night and ensure good sleep.
4. Prevent asthma exacerbations.
5. Minimise side effects from asthma medication.

Preventive and symptomatic pharmacologic therapies in use at the present time are not fulfilling the above mentioned criteria, in addition to being short acting[19]. What is needed is a radical change in the way we think about the therapeutics of asthma[5,7,21,22,24].

Research has continued to evolve both for the perfecting single class of drug and for a better addressed long-term treatment, although physicians have not disposed of new drugs for over 30 years. *Only with regards to chromones expectations have been disappointing. In fact, the absence of an ideal drug determines the use of the drug with the fewest side-effects*[37]. Leucotriene inhibitors, a group of new drugs are useful in mild to moderate asthma[27,41]. Results obtained in stage II and III of this study showed that drug (N) therapy can fulfill most of the goals of asthma management mentioned above. Treating cases of severe chronic asthma using a schedule of 30 capsules total over 12 months, resulted in :

65-100% reduction in symptom scoring,

clinically significant increase in pulmonary function test parameters,

quantitative and qualitative changes in the sputum with more than 70-90% reduction in eosinophil count in the sputum compared to pre-treatment condition.

The development of this hypothesis occurred in 3 stages:

Stage I - Being a consultant clinical immunologist, suffering from chronic severe allergic rhinitis, asthma and laryngeal oedema, I underwent certain self limiting health problem in around October 1993, after which my asthma improved greatly (serial serum samples, nasal smears, pulmonary function tests preserved). This incident led me to think of a possible theoretical linking of drug (N) with allergy.

I was eager to convert it into a practical applicable form. I managed with stupendous efforts, in sympathy with patient sufferings, to keep this research ongoing and to communicate and consults a number of prominent immunologists, locally and abroad finally being enabled to hope to bring the innovation (documenting the value of drug (N) in allergy and asthma) to light.

Stage II - The primary objective of this stage was to determine the usefulness of drug (N) therapy in allergy, as a new therapeutic approach or as an alternative in cases resistant to traditional therapy. 120 adult subjects of age group (20-68) with various acute and chronic type I hypersensitivity were treated with drug (N). In a double blind placebo controlled trial after an informed consent into the study. All the subjects were of matched age, sex, type and severity of allergic condition.

Design of the Study

1. Diseases involved include seasonal allergic rhinitis, allergic conjunctivitis, chronic urticaria, asthma and laryngeal oedema, (Table 1).
2. The duration of treatment, the total dose received and the schedule of therapy were verified to find the best method of treating various allergies.
3. The patients were evaluated daily regarding symptoms severity over the preceding 24 hours[35]. A global overall evaluation of treatment efficacy was made by the doctor at intervals accorded to patients attendance.
4. The response was recorded according to the onset of a noticeable effect and the degree of symptomatic improvement.

Table 1 : Final global improvement rating of patients in stage II.

	No. of patients	Markedly improved	Moderately improved	Slightly improved	Unchanged	Difficult to evaluate	Dropped from the study
Seasonal allergic rhinitis	25	15 (60%)	7 (28%)	3 (12%)			
Allergic conjunctivitis	8		1 (12.5%)	2 (25%)	4 (50%)		1 (12.5%)
Chronic urticaria	6		1 (16.6%)	1 (16.6%)	2 (33.3%)	2 (33.3%)	1 (16.6%)
Asthma	15	9 (60%)	4 (26.6%)	1 (6.6%)	1 (6.6%)		
Laryngeal oedema	6	2 (33.3%)	3 (50%)		1 (16.6%)		
Total	60	26 (43.3%)	16 (26.6%)	7 (11.6%)	8 (13.3%)	2 (3.3%)	2 (3.3 %)

5. All the patients included were having severe symptoms which are sufficiently troublesome to interfere with daily activity or nocturnal sleep.

The final global improvement rating includes[25].

* **Markedly improved:** almost approaching normal condition.
* **Moderately improved:** having mild symptoms.
* **Slightly improved:** having frequently troublesome symptoms but not sufficiently interfere with daily activity or nocturnal sleep.
* **Unchanged:** remain as in the pretreatment condition.
* **Difficult to evaluate:** no conclusion could be reached.

6. Three main symptoms were chosen for each of the conditions studied, they were:

* **In seasonal allergic rhinitis:** running nose, frequency of sneezing, nasal obstruction[28,35].
* **Allergic conjunctivitis:** redness of the eye, itching, swelling[20].
* **Chronic urticaria:** frequency of recurrence, distribution on the body, severity of itching[17].
* **Asthma:** dyspnoea, wheeze, cough[18].
* **Laryngeal oedema:** fullness in the throat, hoarseness of the voice, inspiratory difficulty.

The data collected depending mostly on symptomatic improvement and chronological events, resulted in arriving at a clear idea of the usefulness of the invention as a practical approach in the treatment of these patients. The results of treatment were impressing and having immense advantages over the presently available drugs, being orally administered, for children and adults, simple schedule of treatment, has no sedative effects, no tachycardia or tremor, with few side effects mentioned in the manufacturer's leaflet. During drug (N) treatment it was possible to stop all other forms of therapy, including steroids. Long term prophylactic effect was noticeable.

Bus
PV

In order to get objective evidence of the therapeutic usefulness of drug (N), we proceed to Stage III.

Stage III - Since August 1995, nine patients with chronic severe asthma[3,18], all of whom were on a maximal dose of bronchodilators and maintenance corticosteroids were chosen on account of poor response to conventional treatment, were treated with drug (N), and followed up, till now, as convenient for the patient and feasible in our difficult health service situation with the blockade. Their demographic data are being shown in Table 2.

Design of study:

* Day (0): is considered as baseline. The patients were receiving maximal dose of anti-asthma drugs and corticosteroids.
* Day (1): is the beginning of drug (N) treatment the patients received the recommended oral dose in addition to previous therapy.
* The total amount of drug (N) used is 30 (thirty) capsules, or what is equivalent during the whole study period. They were asked to refrain from taking their conventional drugs when possible.
* The study parameters are presented as Absolute numbers to be compared with pretreatment assessment.

	Age (years)	Sex	Duration of illness (years)	Family History	Type of Asthma	Date of starting treatment	Other allergies	Hypo sensitization	Skin test	ketotifen Intal (DSCG)	other illness	Blood sample available
Nida	42	F	3	+ve	Extrinsic	oct.1993	rhinitis conjunctivitis Larangeal oedema	No	+ve	ketotifen 6 months	No	3
P1 NJ	58	F	32	Father + step son+	Extrinsic	1/8/95 (20 month)	conjunctivitis ear	for 1 year no response	+ve tree pollen penicillin		Diabetis	14
P2 ZK	58	F	32	-ve	Intrinsic disappear on north..	18/10/95 (18 month)	No	some improvement	+ dust mite fish beans	Intal 2 months no response	Diabetis hypertension ischaemic H D	2
P3 FZ	48	F	13	18 year till now 3 sons up to 10 years Father	Extrinsic	12/9/95 (19 month)	chemically Induced	NO	house dust mites	Intal effect similar to puff	Diabetis thyrotoxicosj	3
P4 AS	62	M	25	- ve		4/1/97	- ve	NO	NO	Zaditen	NO	7
P5 HS	45	M	5	+ ve		23/12/96		NO		improve	NO	9
P6 AG	72	M	32	3 daughters	Intrinsic	20/8/95	NO	NO	- ve	NO	NO	1
P7 ZU	48	M	1	- ve	Intrinsic	17/8/95	NO	NO	NO	no response	NO	2
P8 MD	36	F	4	- ve		10/8/96	rhinitis conjunctivitis	NO	NO	Ketotifen useful	NO	
P9 IB	60	M	3	+ ve		14/4/97	NO	NO	NO	NO	NO	5

Table 2 Demographic data of patients involved in stage III

* All patients were advised to stop their corticosteroids and anti-asthma drugs where possible.

Assessment Criteria

1. Symptom scoring:
   a- Symptom triad of dyspnoea, cough and sputum scoring, the maximum score for each is 3. Scores for dyspnea were assessed as: no dyspnoea (score 0), mild on doing physical activity (score 1), moderate at rest (score 2), severe constant annoying dyspnoea (score 3). Scores for cough were assessed as: no cough (score 0), mild cough sometimes (score 1) moderate frequent annoying cough (score 2), severe constant distressing cough (score 3). Scores for sputum were assessed as, no sputum (score 0), small amount expectorated with ease (score 1), tenacious moderate amount (score 2), plenty causing severe mucus-related symptoms (score 3).
   b- Composite symptom scoring: this is an indication of therapeutic effectiveness in improving the patients global assessment[25,30]. The sum of the score is 39, three is the maximum for each symptom, which includes cough frequency, cough severity, ease in bringing up sputum, audible wheeze, tachycardid, chest discomfort, nocturnal dyspnoea disturbing sleep, ability to go upstairs, ability to talk and laugh, stress incontinence caused by the cough, missing days at work, psychological well being. hospitalization rate and need for I.V. drugs.
2. Five patients were able to do serial complete pulmonary function tests (PFT). Using Autospiror (Discom 14, Chest Corporation, Tokyo, Japan). P.F.T. before starting drug (N) therapy was considered as a base line. Daily when possible during the first week and according to patients attendance later on, aiming at finding whether drug (N) is having a bronchodilating effect by evaluating the changes in forced expiratory volume 1st second (FEV1), peak expiratory flow rate (PEFR) at 25%, 50% and 75% of vital capacity (FEF 25%, FEF 50%, FEF75%).
3. Serial microscopical sputum examination for the percentage of eosinophils in relation to other inflammatory cells. Samples were smeared on slides, fixed with methanol, stained by haematoxylin - eosin. A total of 300 inflammatory cells were counted in each slide and the percentage of eosinophil calculated (slides are preserved).
4. Serial serum samples kept at -20 °C, awaiting reagents availability to carry out certain relevant Laboratory tests as total serum IgE (PRIST, Pharmacia, Upsala, Sweden), Ostecalcin (Pharmacia) to follows the systemic effect of corticosteroid treatment, Eosinophil Cationic Protein (Pharmacia) to measure eosinophilic inflammation, follow the efficacy fo drug (N) treatment and monitoring patients compliance, and others.

Results:

1. Symptom scores:

a- Symptom triad: the three major symptoms of dyspnoea, cough and sputum were improved by 67-100%. The improvement started by day 3, to reach maximal within 7-10 days, as shown in figure (1-3 and 10-12). The improvement lasted over the whole study period, with few mild attacks of short duration.

b- Composite symptom scores: the score were improved by 67-100%. The improvement started by day 3, to reach maximal within 7-10 days. As shown in Figures (4 and 13). The improvement lasted over the whole study period with only few mild attacks. They are now free from multiple drug therapy, breath better, leading more comfortable lives, and staying out of the hospital.

2. Changes in pulmonary function test (PFT) parameters:

The bronchodilating effect (more correctly, alteration in airway flow and bronchial patency, resulting from drug (N) therapy, was evaluated as absolute changes in FEV1, PEFR, FEF 25%, FEF 50%, FEF 75% over base line during a period (20-25) days Figs.(5-9) and (14-18) and according to patients attendance later on.

P3: FEV1 increased by 39.7%, range 4.7% - 53.76%) (Fig. 5).
increment in FEF 25% was 49.73%, range (30.0% - 63.16%) (Fig. 9).
increment in FEF 50% was 56.83%, range (0.0% - 66.04%) (Fig. 8).
FEF 75% increased by 38.04%, range (29.41% - 46.96%) (Fig. 7).

P2: increment in FEV1 was 18.09%, range (8.69% - 22.22%) (Fig. 5).
FEF 25% increased by 22.84%, range (-15.38% - 40.0%) (Fig. 9).
The increment in FEF 50% was 34.17%, range (12.82% - 49.25%) (Fig. 8).
The increment in FEF 75% was 63.39%, range (56.25% - 68.18%) (Fig. 7).

P1: increment in FEV1 was 4.42% (range 2.32% - 6.67%) (Fig. 5).
increment in FEF 25% (alveolar) (Fig 9) was 43.49%, range (3.33% - 59.15%).
FEF 50% (small airways) (Fig 8) increased by 26.65%, range (-5.75% - 44.24%).
FEF 75% (large airways) (Fig 7) increased by 43.81%, range (6.45% - 55.16%).
Clinically significant changes in PEFR were also noticed.
Patients 8 and 9 are also presented.
The changes are maintained during the whole study period.

3- Sputum changes:

Macroscopically there was a tremendous decrease in the amount of sputum, it became thin easily expectorated, changes started by day 2 and within 5-7 days the patients were free of mucus related symptoms.

The percentage of eosinophils to other inflammatory cells decrease from 80% to less than 10% within the 1st two weeks.

4-Changes in the serum during the study period need to be unmasked later, after carrying the appropriate laboratory tests.

Long Term Follow-Up

The patients involved in stage III had been followed-up for varying periods starting Aug. 1995. The total dose of drug (N) received is thirty (30) capsules or what is equivalent, no additional dose was given.

The assessment criteria include: 1. Rate of hospitalization, 2. The need for concomitant traditional therapy, 3. The frequency of attacks of shortness of breath, cough, wheeze and sputum, 4. Daily activity, 5. Disturbance of sleep.

The follow up showed that the hospitalization rate was reduced from several times *per month* to 1-3 times *per year*, they needed concomitant therapy when they got a cold only, the asthmatic attacks we very few and lasted for a shorter time and were much milder ,manifested mainly as shortness of breath, mild cough, *scanty or no sputum.* Eight of ten were able to live a rather normal life, night and early morning dyspnoea disappeared, no mortality was recorded among them.

The sputum is absent all the time and only very scanty during acute exacerbation. Microscopical examination showed that the reduction in eosinophils number / compared to other inflammatory cells was 5-10% and was maintained except during exacerbation where it rises to 30-40% for a short period.

Serum samples taken at varying intervals were kept in (-20 °C).

It had been noticed that drug (N) treatment got a preventive and long term effect, particularly in seasonal allergic rhinitis, laryngeal oedema and asthma and might lead to a new hypothesis in the immunopathogenesis of type I hypersensitivity.

Discussion:

It is well known that asthma is a common disease. A wide spectrum of anti-asthma drugs are available, some are treating the symptoms, and others aiming at the immunopathologic factors involved. Any of these drugs, singly or in combination, are not fulfilling the goals of asthma management[18] in many patients. A continuous search for a new better drug is going on[4,5,12,22,24,26,27]. *The ultimate test for the therapeutic effect of a new drug is clinical trial*[27].

In our present hypothesis, after the theoretical linking of drug (N) with allergy, a clinical trial was carried out, to evaluate the therapeutic effect and reducing it to practice. During stage II we depend mainly on subjective improvement and chronological events. The results were greatly impressing. In stage III, 9 patients were treated by drug (N) according to a schedule of 30 capsules in total the assessment criteria were subjective improvement in addition to studying the effect on objective parameters. Assessment involves.

1. Symptom scoring

a- Symptom triad of the three cardinal symptoms, dyspnoea, cough and sputum.

b- A more comprehensive global self assessment scoring involving 13 symptoms (composite symptom score)[20,25].

The results of both assessments was a 65-100% reduction in the score started by day 3 to reach maximum in day 10 and was maintained during the study period of 13-10 months. A search in database (MEDLINE silver platter 3.11) up to May. 1997 showed no similar therapy, therefore, such results are novel.

2. The second assessment criteria was a complete pulmonary function test, to assess airway patency and detect "how much" of a bronchodilating effect does drug (N) treatment produces.

FEV1 is a valuable screening procedure for obstructive lung diseases. It is also useful in assessing the efficacy of bronchodilator therapy and in following the progress of the patient with asthma, 15% increase in FEV1 over baseline is considered clinically significant[2,15]. In our patients the percent increment in FEV1 over the base line, during the 20 days period (Fig. 5) in more than 15%, so it has got a significant bronchodilatating effect. Changes actually started after 4-6 days therefore, it seems that the increment in FEV1, the alteration in airway flow and bronchial patency is the result of an effect on the basic immunologic inflammatory process.

In P3 FEV1 increment was evident by day 4 (57.47% increase from base line and maintained later, but the day 2 reading (4.76%) lowered the mean.

Regarding P2 mean FEV1 was lowered by delay onset of action and by a low reading by day 7 (4.55% increase from base line) due to the natural course of the disease. Otherwise the % increment was around 20% patient was off- corticosteroids and continued to need bronchodilator tablets and puffs on and off.

In P1. FEV1 base line value was very high (more than 75% of predicted) and was maintained during the study period. There was a marked lowering by day 4 (-13.51), which Negatively effect the mean FEV1 increment, otherwise, it will be about 22.28%.

The increment in forced expiratory flow at 25% of vital capacity (FEF 25%) (alveolar), FEF 50% (small airways), FEF 75% (large airways) was tremendous. An increase of 20-25% from base line is considered clinically significant. The results in the patients were about or, 2-3 folds, this value. Clinically significant PEFR changes were also observed. Patients 8 and 9 showed similar changes.

Together with the other highly positive changes in the various assessment criteria, is reflecting a possibility that their is a gradual switching-off of the inflammatory process. *Such increment in the various parameters is totally unexpected.*

3. Sputum changes:

Hypersecretion of mucus causing mucus related symptoms (25) and changes in its viscoelastic properties, are characteristic of asthma. During drug (N) treatment there were a marked decrease in the quantity and a change in viscoelastic properties, clinically evident by 3 days. Most of mucus related symptoms disappeared by day 10.

Eosinophils are a key inflammatory cells in asthma, it is pathologically considered as a chronic desquamative eosinophilic bronchitis. The number of eosinophils in the sputum is correlated to the severity of the disease. Ellinor A. et. al.[9] has shown that the total cell number, or differential cell counts in either the bronchial wash or the bronchoalveolar lavage fluid, before and after treatment with budesonide or terbutaline, showed no significant chanes. Patricia D. et. al.[25] had shown that 28 days treatment with cromolyn 40 mg (two spincaps) four times daily, resulted in a significant decrease in the percentage of eosinophils in bronchial mucus in the responder group. Zaditen (Ketotifen) is inhibiting the accumulation of activated eosinophils[30].

In this study sputum examination shows that there was more than 70-90% reduction in the number of eosinophils related to other inflammatory cells. Upon correlating the reduction in the number of eosinophils per unit volume of sputum, with the daily sputum output, the reduction in eosinophil number was remarkable. Such results bear great similarity to drugs that are treating the underlying cause of asthma, and will possibly lead to a new approach in the immunopathogenesis of asthma.

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I would like to thank *Dr. Akram Abbood* for statistical help, Dr. Muna Taki for sputum examination, Mrs. Nahida Al-Janabi for PFT, and Mrs. Najat Mihsin, Faiza Ali, Zakia Khalid for cooperation.

N. Nazzal

Sincerely Yours

Online Articles

Nigella Sativa, Commonly Known As "Love in the Mist" A Beautiful Middle Eastern Herb With Many Uses

Dr. Michael Tierra L.AC., O.M.D.

Nigella
(NIGELLA SATIVA L.) Black Cumin, Fitch (Biblical), Love in the Mist, Fitches
"...For the fitches are not thrashed with a threshing instrument. ..but the fitches are beaten out with a staff..." Isaiah 28

- Parts Used: seeds
- Energy and Flavors: Hot energy, spicy flavor
- Systems Affected: Lungs, Stomach, spleen
- Biochemical Constituents: Alanine, arginine, ascorbic-acid, asparagine, campesterol, carvone, cymene, cystine, dehydroascorbic-acid, eicosadienoic-acid, glucose, glutamic-acid, glycine, iron, isoleucine, leucine, d-limonene, linoleic-acid, linolenic-acid, lipase, lysine, methionine, myristic-acid, nigellin, nigellone, oleic-acid, palmitic-acid, phenylalanine, phytosterols, potassium, beta-sitosterol, alpha-spinasterol, stearic-acid, stigmasterol, tannin, threonine, thymohydroquinone, thymoquinone, tryptophan, tyrosine
- Properties: Stimulant, aromatic, carminative, digestive, diuretic, emmenagogue, excitant, galactatagogue, purgative, resolvent, stimulant, stomachic, sudorific, tonic, and vermifuge

Uses: For me the common name "love in the mist" aptly describes the poetry of this exquisite plant. In the garden, one easily imagines etheric spirits flitting about amongst its evanescent bluish-white blossoms. Even the seedpods, which are so often used in dried flower arrangements, suggest an otherworldly sense of exotic enchantment. Is it possible that such a delicately beautiful herb, with such potent medicinal properties would be so hardy as to easily reseed itself in our gardens year after year?

With an exalted position of use throughout the Middle East and to a somewhat lesser extent in India and other Eastern lands, the information about Nigella I owe to herbalist, plant-scientist extraordinaire, Jim Duke as presented in his book Medicinal Plants of the Bible. In it he describes Black Cumin as a Muslim Miracle Herb which, according to an Arab Proverb it is said that, 'in the black seed is the medicine for every disease except death.'

I have spoken with a Turkish colleague who reports that it the seeds are widely cultivated and traded in ton lots within his country throughout the Middle East, Northern Africa and India. The seeds are used both as a condiment in bread and cakes and various confections and like pepper or combined with pepper such as cayenne in sauces. The Ethiopians add along with other spices to flavor local alcoholic beverages. Still another use is to sprinkle them with woolen garments as a moth repellant.

The major uses I have employed it for are upper respiratory conditions, allergies, coughs, colds, bronchitis, fevers, flu, asthma and emphysema for which it is effective. Simply collect the abundance of seeds from the pods and grind them to a paste and mix with melted honey to a 'hahlava' (a Middle Eastern confection usually made with toasted sesame seeds and honey). Jim Duke confirms its folk use for these and a wide variety of other diseases and conditions including bilious ailments, calluses, cancer, colic, corns, eruptions, headache, jaundice, myrmecia, orchitis, puerperal fever, sclerosis, skin, snakebite, stomachache, swellings, tumors of the abdomen and eyes, and warts. In Algeria, the roasted seeds are combined with butter for cough and honey and taken for colic.

2A

For upper respiratory conditions, at least a few of its constituents have shown an antihistamine-like action, which explains is positive effects for upper respiratory diseases including asthma, bronchitis, and cough. The oils of the seed increase milk flow which explains its folk use as a galactagogue. In large quantities, however, the seeds have also been used to abortion.

It is unusual for a hot spicy herb to have a positive effect on liver diseases as it is used by the Lebanese. Of course, one of its most obvious uses is for diarrhea and dysentery, combined with astringents. Externally the seeds can be ground to a powder, mixed with a little flour as a binder and applied directly to abscesses, on the forehead for headache, nasal ulcers, orchitis, and rheumatism. The seeds also are a rich source of sterols, especially beta-sitosterol, which is known to have anticarcinogenic activity. This substantiates its folk use for indurations and/or tumors of the abdomen, eyes and liver.

In India, Nigella seeds are combined with various purgatives to allay gripping and colic and also help kill and expel parasites. Middle Eastern Unani medicine affirms its abortifacient properties and also use it as a diuretic to relieve ascites, for coughs, eye-sores, hydrophobia, jaundice, paralysis, piles and tertian fever.

Contraindications: Do not take during pregnancy.

2B

A SEARCH FOR AN INDUSTRIAL PARTNER IN DEVELOPING, PATENTING AND MARKETING A NEW ANTI-ASTHMA, ANTI-ALLERGIC DRUG.

Inventor: Dr. NIDA NASSIEF, MD, M.Sc (U.K.)
Consultant Clinical Immunologist and Pediatrician
Central Public Health Lab. Baghdad-IRAQ

Associate Workers: -

DR. M. N. Mohammed, MD, M.Sc., Ph.D. (U.K.), Asst. Prof. of Medicine, Mustansiriya University.Baghdad –Iraq .

DR. Z.A. FADHEL, MD, DVD (U.K.), Consultant Dermatologist. Yarmook Teaching Hosp.

DR. H.A.G. NASSIEF, MD, D.O., FRCS (U.K.), Asst. Prof. in ophthalmology .Mustansiriya University.

Back during October 1993, certain health incident happened to the inventor, after which, her asthma and laryngeal oedema was tremendously improved. Based on this event, a theoretical speculation of the immunological events that resulted in the improvement was forwarded. Conceiving the idea of the whole research was considered as STAGE I.

Since then, stupendous efforts motivated by patients suffering are continuous to complete the work.

STAGE II: An animal experimentation was designed to make-true one aspect of the theoretical speculation. Four white albino rats were challenged locally intranasaly with certain substance, that is considered as a backbone in the skeleton of the theory .The results of histopathological examination of the nasal cavity of the rat were encouraging, it showed evidence of an *in-vivo* novel eosinophil chemotactic factor. It was concluded that these key inflammatory cells in asthma are related in-a-way to the backbone above mentioned. Fig.1 and fig.2 demonstrates eosinophilic infiltration of rat nasal cavity and submucosa.

STAGE III: A drug (N), present in the market, indicated for diseases unrelated to type 1 hypersensitivity, was linked (depending on the above speculation) with allergy in a novel way, using it in a non-routine indication and dosage.

In order to prove its utility and reduction to practice, a double-blind placebo controlled clinical trial, involving 120 subjects (60 patients treated with (N) and 60 patients matched for age, sex, and severity of the allergic condition were treated with placebo) to assess (N) beneficial

therapeutic effect in various acute and chronic allergies, depending on symptom scoring and chronological events.

1.Diseases involved includes seasonal allergic rhinitis, allergic conjunctivitis, chronic urticaria, and asthma and laryngeal oedema.

2.The duration of treatment, the total dose received and the schedule of therapy were verified to find the best method of treating various allergies.

3.The patients were self-evaluated daily regarding symptoms severity over the preceding 24 hours. A global overall evaluation of treatment efficacy was made by the doctor according to daily notes at intervals, depending on patients attendance.

4.The response was recorded according to the onset of a noticeable effect and the degree of symptomatic improvement.

80% of the treated patients showed totally unexpected marvelous symptomatic improvement particularly seasonal allergic rhinitis, asthma and laryngeal edema.and a long-term effect were noticed, hence proceeding to: -

STAGE IV: in which, a study was designed to *objectively* evaluate the therapeutic effect of (N). Since Aug. 1995, nine severe chronic asthmatics receiving maximal doses of anti-asthma drugs and corticosteroids, chosen on acount of poor response to conventional treatment were treated with (N), administered orally according to a schedule of 30 capsules (or what is equivalent) in total, over the whole study period, while continuing their previous therapy. Assessment was carried out by using:

- Symptom scoring

  One-Symptom triad of the three cardinal symptoms, dyspnoea, cough and sputum.

  b-A more comprehensive global self-assessment scoring involving 13 symptoms (composite symptom score).

The results of both assessments was a 65-100% reduction in the score started by day 3 to reach maximum in day 10 that was maintained during the study period of several months to few years. The patients were enabled to lead a rather normal lives, with minimal convential anti asthma drugs and odiscontinue corticosteroids, Fig.3, Fig.4 shows the pattern of changes in symptom scoring.

- The second assessment criteria was a complete pulmonary function test, to assess airway patency and detect "how much" of a bronchodilating effect does drug (N) treatment produces. FEV1, PEFR, FEF 25%, 50% and 75% are valuable screening parameters for obstructive lung diseases. They are useful in assessing the efficacy of bronchodilator therapy and in following the progress of the disease.

Drug (N) therapy results in a clinically significant increase in parameters reflecting obstructive lung disease. The amplitude of the changes are greater than what is produced by a bronchodilator drug.

- Sputum changes: During drug (N) treatment there were a marked decrease in sputum quantity in addition to a change in viscoelastic properties, clinically evident by 3 days. Most of mucus related symptoms disappeared by day 10.

Serial microscopical sputum examination shows that there was about 70-90% reduction in the number of eosinophils related to other inflammatory cells reached by day (14). Upon correlating the reduction in the number of eosinophils per unit volume of sputum, with the daily sputum output, the reduction in eosinophil number was remarkable. Such results bear great similarity to drugs that are treating the underlying cause of asthma. Fig.5 and fig.6 demonstrate the reduction in sputum eosinophils.

- Serum changes: Serial serum samples from patients were taken and stored at (-20) awaiting reagent's availability to unmask the changes in relevant parameters.

Serial samples from stage I are also stored.

A NEW HYPOTHESIS in the immunopathogenesis of type I hypersensitivity were built up.

The corner stone is the tremendous reduction in sputum eosinophils that was maintained for very long period, temporarily abolished during acute exacerbation. It occurs even in none-responder group.

Other stones of the new hypothesis are:

- The delayed onset of action.
- The pattern of the changes in symptom scoring.
- The amplitude of changes in the relevant parameters of pulmonary function test.
- Long term effect (years).
- The response of both extrinsic and intrinsic asthma

All of them indicate a novel unpreceded-till now-*selective switching off* of the eosinophilic airway inflammation. Hopefully this will unmask important scientific facts in the immunopathogenesis of allergy and asthma that will at the end pave the road for a new antiasthma era.

Information search regarding patent laws showed that there is an Australian law that applies to patenting new medical treatments. (Louphlan P.L; Med. J. Aust. (1995) 3: 162). Therefore, during early 1997, the Australian Industrial Property Organization (AIPO) lit a candle in the research way. Their opinion stated that "it appeared from the details you provided, that you have sufficient experimental results for a patent application". Their advice was to contact the World Intellectual Property Organization (WIPO). Copies of the research results were sent to WIPO and the Department of Health and Human Services, Food and Drug Administration (FDA) for evaluation. Thankfully, FDA forwarded the copy to the center for drug Evaluation and Research for review as a New Drug Application (NDA) <u>without</u>

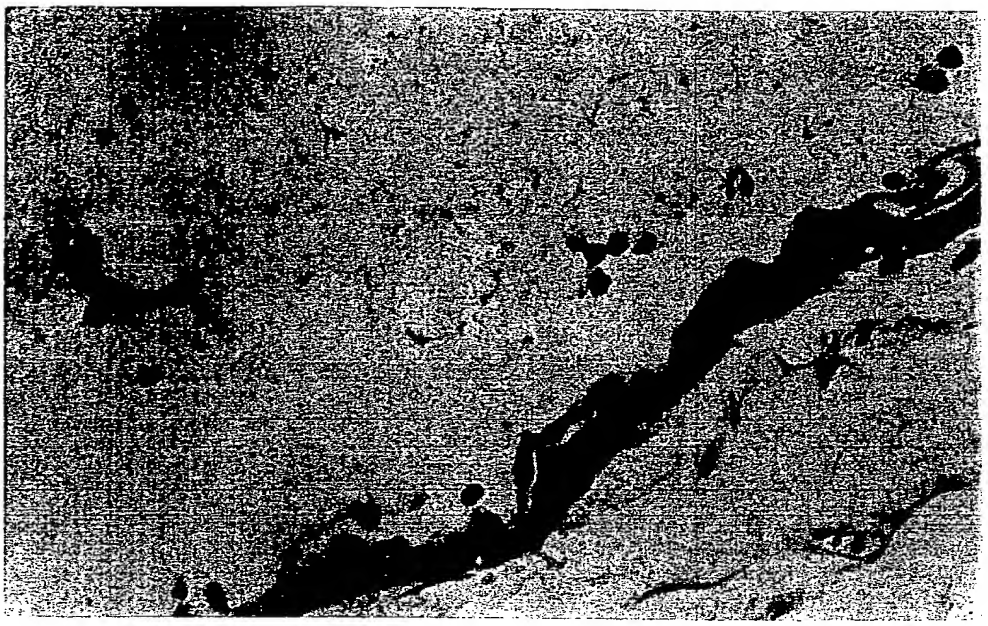
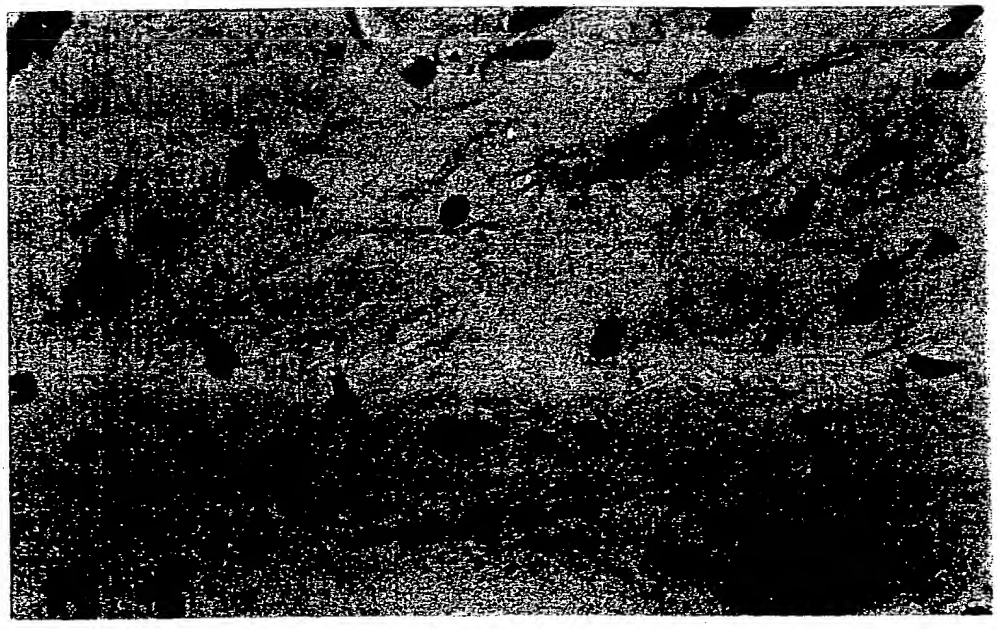


Fig. 1, above : A microscopical view of a section of rat nasal cavity and mucosa stained with haematoxyline –Eosin (H & E) magnification X 400, showing evidence of eosinophil chemotaxis (arrowed).

Fig. 2, below : Eosinophil infiltration of the submucosa.



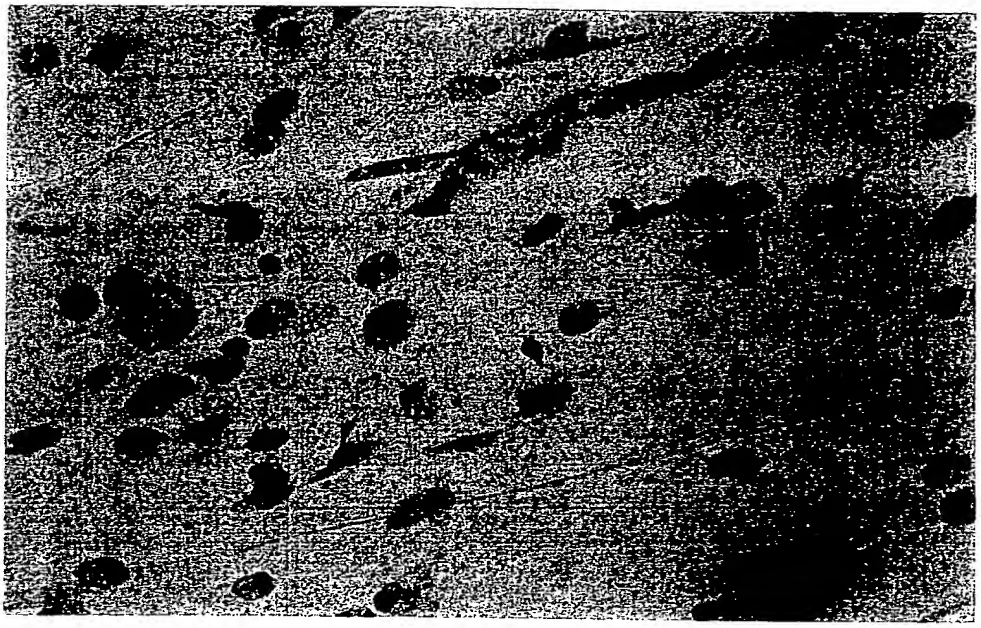


Fig. 5, above : Microscopical view of patients sputum H & E stained X 400, *befor* drug (N) therapy , The field is full of eosinophils , *Owl eye like appearance* .

Fig. 6, below : Same patients sputum , *after* treatment,There are very scanty eosinophils and reduction in inflammatory cells. Carbon–Laden macrophages and fibroblasts are seen.

unmasking Drug (N) identity. A patent attorney was consulted, and after a careful search, he found that the invention is novel.

After all these stupendous self supported efforts of years we are still far away from providing this miracle in-between the hands of the suffering patients, while being able to preserve our rights.

STAGE V: since late 1997 and during 1998 efforts were continuous to develop an **ALTERNATIVE SUBSTANCE (A)**, which can be patented, has it's own identity and trade name, in order to replace drug (N). Before hand the immunological action of substance (A) was studied in vitro. Its beneficial effect to allergy was accidentally noticed. Therefore, depending on these informations, a clinical trial was started during July 1998.The available clinical data showed similarity of anti-asthma effect of the alternative substance (A) with the previously used drug(N).

Substance (A) has got the following characters:

1. It is orally administered in a simple schedule similar to drug (N).
2. There is evidence that (A) or its metabolites are excreted in the urine.
3. Some aspects of its mode of action has been studied in vitro.
4. Is considered to be safe as it had been used by human beings since the ages.
5. It is not registered under any trade name, most likely it will be a patentable invention.
6. It seems that *the secrete lies in the schedule of therapy* as well as in the mode of action of substance (A) and drug (N).

At present we are in urgent need for an industrial partner to take over the completion of the invention and sponsoring patenting a marketing. With my best regards and hopes for dual cooperation.

Acknowledgments:

I would like to thank Dr. Muna Taki for sputum examination, Mrs. Nahida Al-Janabi for PFT, Dr. Nabil Abu-Zaid for lab. Assessment of substance (A),Dr. *Akram Abbood* for statistical help, Dr. Najah Mohd Ali, and other helping colleges.

Mrs. Najat Mihsin, Faiza Ali, Zakia Khalid, and Mr.Abdul-satar for cooperation.

Sincerely Yours

N. Nassir

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Although a variety of changes can be measured, lymphocyte responsiveness is most commonly assessed by measuring DNA synthesis through the incorporation of radiolabeled thymidine (3).

And it has been found that the amount of DNA synthesis correlates with the percent of cells becoming blastlike. DNA synthesis is measured by the incorporation of 3^H-thymidine into water-insoluble nuclear material.(Ref)

The radioactivity increases in proportion to the number of lymphoblasts formed in culture, hence only a simple calculation remain be done.(Ref)
The stimulation index: is the relative increase in 3^H-thymidine incorporation into DNA the the presence of mitogen and antigen compared to the control without the addition of mitogen and antigen. The stimulating index is50-200 for mitogens and over 3 for antigen.

Preparation of substance (A) extracts

For lymphocyte stimulation with Mitogen and antigen lab. test.

- 3.5 gram of substance (A) was finely grinded to a powder.
- In a volumetric buret, 100cc chloroform and then 100cc distilled water were placed. Then substance (A) powder was added and the buret was capped.
- Manual shaking was performed for ½ hour.
- It was hanged in a buret holder overnight.
- Next day the suspension was settled into separate layers inside the buret as shown in the drawing. Each layer is going to be tested separately for it's ability to stimulate lymphocyte culture.

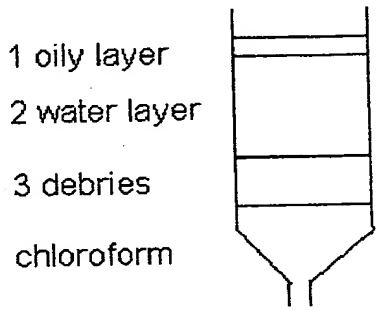

Fig. 1: showing layers formed during extraction of substance A, in the buret hanged overnight.

- Each layer was collected in a different container and kept at 4℃
- The test was performed after 10 days.

Procedure:

1. All cell manipulation was performed using sterile techniques and at room temperature.
2. Blood sample was drawn fresh by venipuncture and anti coagulated with 20 IU heparin /ml from healthy normal females 30-45 years of age.
3. Whole blood was layered gently on 15 ml lymphoprep trying not to disturb the interface then the tube was capped.
4. Centrifuge at 1500 RPM for 30 minutes at 18□C.
5. With a Pasteur pipette and the attached bulb depressed the plasma was discarded. The lymphocyte band removed to be used in the following steps.
6. The lymphocytes was washed with phosphate buffers saline (PBS) and resuspended to a final concentration of 1X10^6 cell concentration /ml in RPMI medium containing penicillin and streptomycin.
7. 0.1 ml of cell suspension was added to *triplicate* wells of microculture plate.

CO_2 incubator.

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1. Antigens and mitogens were added to different plates in triplicates as follows:
   - PHA mitogen at concentration of 0,50,100,150,200,250 microgram /ml.
   - PPD antigen at concentration of 0,10,20,30,40,50 microgram /ml.
   - Substance (A) water extract diluted from stock to ½ , ¼ , 1/8 , 1/16 , 1/32.
   - Oil extract

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Mean CPM	Normal Control(5) CPM	Normal Control(4) CPM	Normal Control(3) CPM	Normal Control(2) CPM	Normal Control(1) CPM	Conc. Microgm/ml
8395	8290	7799	8558	8438	8888	0
37449	40100	40318	37530	35482	33814	50
46159	49277	54666	45310	44318	46224	100
* 60611	59347	65267	59795	58347	60298	150
53788	51027	55821	55810	54230	52052	200
43126	39400	40500	40324	49722	45686	250

Table(1)

Mean CPM	Patient (5) CPM	Patient (4) CPM	Patient (3) CPM	Patient (2) CPM	Patient (1) CPM	Conc. Microgm/ml
6930	7060	6821	6992	6855	6922	0
33107	32254	36620	34999	31438	30222	50
41440	39274	40210	42371	41786	43558	100
*55749	58002	54029	54102	55332	57279	150
47236	44382	42720	45200	51827	49050	200
41237	38424	39055	44111	43385	41211	250

Table(2)

Dose - response table for mitogen (PHA) stimulation of 10 ^6 lymphocytes

Lymphocytes were pulse – labeled with 2 microcuri of tritrated (H^3) thymidine 20 hours prior to harvesting, counts per minute (CPM) were determined by liquid scintillation spectrometry .

Maximum response occure at 150 microgm/ml

Day	Normal control (1) CPM	Normal control (2) CPM	Normal control (3) CPM	Normal control (4) CPM	Normal control (5) CPM	Mean CPM
0	8104	7999	8220	7550	8040	7983
1	20830	18580	20320	19855	21452	20208
3	60298	58347	59795	65267	59347	60611*
5	36807	35815	37125	32380	30682	34562
7	21892	24722	25340	19005	18772	21946

Table(3)

Day	Patient (1) CPM	Patient (2) CPM	Patient (3) CPM	Patient (4) CPM	Patient (5) CPM	Mean CPM
0	6010	6064	6090	6992	6927	6412
1	17155	16282	17870	20834	16650	17758
3	57279	55332	54102	54029	58002	55749*
5	33125	36222	34672	30604	30110	32947
7	20621	18046	19696	16500	18644	18741

Table(4)

Time - response table for mitogen stimulation (PHA) or 10^6 Lymphocytes.

Peripheral blood lymphocytes using an with an optimal concentration of (PHA) (150 microgm/ml).

Maximal response occured at (3) days after initiating .

Conc. Microgm/ml Of PPD	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8044	7915	7866	8022	7890	7947
10	14189	13885	13262	14552	13830	13944

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20	26063	24660	24322	23380	26608	25007
30	38115	36239	36811	38404	43750	36864*
40	28362	25466	25444	28755	27358	27077
50	20069	19920	20508	22210	19250	20391

Table(5)

Conc. Microgm/ml PPD	Patient (1) CPM	Patient (2) CPM	Patient (3) CPM	Patient (4) CPM	Patient (5) CPM	Mean CPM
0	7814	7855	8020	7944	7828	7892
10	13208	12332	13642	13928	12686	13159
20	25882	24246	23676	23782	26993	24916
30	36446	34313	35267	35320	34875	35244*
40	27351	28360	25984	28262	26989	27389
50	20485	20230	19590	21623	18125	20011

Table(6)

Dose-response table for antigen stimulation (PPD) at 10^6 lymphocytes.

Culture were harvested for 120 hours at culture after 20 hours with tritiated (H^3) thymidine counts per minut (CPM) were determined by liquid scintllation spectrometry.

maximum response is at 30 microgm/ml of antigen.

Day	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8000	7774	8022	8004	7908	7942
1	14351	12556	13814	12500	13212	13287
3	26682	22111	26823	20251	24815	24267
5	38115	36239	36811	38404	34750	36864*
7	25166	23750	29402	28987	26223	26666
9	19758	21482	22650	20829	18860	20716

Table(7)

Day	Patient (1) CPM	Patient (2) CPM	Patient (3) CPM	Patient (4) CPM	Patient (5) CPM	Mean CPM
0	7914	8366	7390	7801	7729	7840
1	13828	11680	13362	13318	12892	13016
3	22416	21120	22444	19755	22365	21620
5	36466	34313	35267	35320	34875	35248*
7	26069	24920	25508	27538	27820	26371
9	15622	19414	20282	19620	19351	18858

Table(8)

Time-response table for antigen stimulation (PPD) of 10^6 lymphocytes.

Antigen concentration for all cultures was optimal concentration (30 microgm/ml).

Maximum response occurred on day (5) of culture .

Dilution of water extrac.	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	7914	8366	7210	7001	7724	6743
1/32	13828	11680	13362	13318	12892	13016
1/16	22416	21120	22444	19755	22365	21620

1/8	36466	34313	35267	35320	34875	35248 *
1/4	26099	24920	25508	27538	27820	26377
1/2	15622	19414	20282	19620	19351	18858

Table I

WATER EXTRACTION

Dilution of oil	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	7914	8366	7210	7001	7724	7643
1/32	13984	14282	11989	12485	14230	13394
1/16	24590	22623	26125	22450	24687	24096
1/8	35897	33389	34391	35302	33155	34427*
1/4	26260	26118	26620	27827	25033	26372
1/2	19250	21623	19357	20820	16755	19621

Table II

OIL EXTRACTION

Dilution of (x)	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	7914	8366	7210	7001	7724	7643
1/32	12355	11382	13141	10500	12382	11952
1/16	24026	22630	21435	20702	22350	2229
1/8	34153	33455	33897	35389	35380	34455*
1/4	24977	22750	22370	23268	24662	23605
1/2	17275	18320	19779	19233	20631	19048

error

Table III

(X) EXTRACTION

Dose response table for substance a stimulation of 10^6 lymphocytes
Maximum response occures at (1/8) dilution of the stock extract.

Day	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8000	7774	8022	7908	8004	7942
1	13208	12332	13642	13928	12686	13159
3	25882	24246	23676	23782	26993	24916

5	36446	34313	25267	35320	34875	35244
7	27351	28360	25984	28262	26989	27389
9	20485	20230	19590	21623	18125	20011

Table IV

WATER EXTRACTION

Day	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8000	7774	8022	7908	8004	7942
1	14354	12351	12225	12894	11262	12617
3	20311	21230	24709	20174	23248	21934
5	35897	33389	34391	35302	33155	34427
7	24211	21150	23830	25844	25056	24018
9	16617	18250	19262	17692	18450	18054

Table V

OIL EXTRACTION

Day	Control(1) CPM	Control(2) CPM	Control(3) CPM	Control(4) CPM	Control(5) CPM	Mean CPM
0	8000	7774	8022	7908	8004	7942
1	11004	12131	12222	11623	12357	11867
3	20820	19755	23355	20382	22141	21291
5	34153	33455	33897	35389	35380	34455
7	25628	24265	25632	28623	26122	26054
9	16253	18048	19225	19220	17852	18120

Table III

CHLOROFORM EXTRACT

Dose response table for substance astimulation of 10^6 lymphocytes. Maximum response occures at (1/8) dilution of the stock extract.

time response

LAIMS DESCRIPTION OF THE INVENTION

A NEW ASTHMA THERAPY THAT ACT ON EOSINOPHILS AND/OR T LYMPHOCYTES

① NOVEL USE OF A NATURAL HERB NIGELLI SATIVUM IN PREPARIN CAPSULES OR SUSPEN: SION FOR ASTHMA AN ALLERGY TREATMENT IN HUMANS + OTHER INDICATONS (Page 2)

T- LYMPHOCYTE STIMULATION TEST SHOWED THAT NIGELLA IS A T- LYMPHOCYTE STIMULANT. IT IS A NOVEL IMMUNO MODULATOR.

② THE USE OF GLICOFOSFOPEPTICAL IN PREPARING AN ASTHMA AND ALLERGY DRUG USING IT IN A NOVEL SCHEDULE OF THERAPY OF 5-20 DAY ONLY (FIVE — TWENTY days only) (AN IMMUNOMODULATOR WHICH IS MARKETED)

③ THE SHORT TERM USE OF ALL IMMUNOMODULATORS IN ASTHMA

[redacted]

Measurements of Lymphocyte activation and proliferation after stimulating them by substance (A) extracts, comparing it to know Antigen and Mitogen.

Abstract:

Measurement of lymphocyte activation and proliferation is an invitro technique for the measurement of cell mediated immunity.

The proliferatine capacity of lymphocytes is measured by their ability to incorporate tritiated thymidine, the newly synthesized DNA in culture. The radioactive is measured by liquid scintillation spectrometer various Mitogens, Antigens, Cytokines or Antibodies are used as a stimulant .

In this study purifide protein Derivitine (PPD), Phytohaemagglutination (PHA) and substance (A) extracts were used as stimulants.

A dose response test to determine the optimal dose for stimulation of this batch lymphocytes .then this optimal dose is used in a time response to detect the time need for maximal incorporation of H3 thymidine .

Results of this study had shown similarity of oil, water, and chloroform extracts of substance A with PPD.

Introduction:

Lymphocyte stimulation is an in vitro technique that commonly is used to assess cellular immunity in cancer, infectious diseases, immunodeficiency and autoimmunity. It was first reported by by Nowel in 1966*. Lymphocytes are stimulated in vitro to become metabolically active by antigens (by antigen) or mitogens. Cell division results in increased DNA synthesis, and 3H-thymidine incorporation (REF. 1) often is used as an indicator of that synthesis.

The ability of substance (A) to activate lymphocytes in culture was studied. Extracts proliferation and lymphocytes transformation is measured by the incorporation of tritiated thymidine in the newly synthesized DNA. The radioactivity increases in proportion to the number of lymphoblasts and can be measured by liquid scintillation spectrometry. Results showed that water, oil and X extracts from substance (A) has got a stimulatory effect on lymphocytes in culture. Similar to PPD, which is an Antigen that can be used for immunotherapy in humans.

This technique consists of placing cultured lymphocytes *in-vitro* in contract with a known concentration of mitogen, or antigen to which they might be sensitive. If the reaction is positive, the lymphocytes is transformed into lymphoblast (6).

An in vitro correlate of cell mediated immunity (CMI).

Is a determination of T-cell proliferation.** This method evaluates the capacity of T-lymphocytes that have been primed in vivo to respond in vitro after culture with the appropriate antigen.

The first step in this response is the interaction of antigen specific T-cells with antigen-presenting cells. After recognition of the specific antigen, the T-cell undergoes a series of physiologic changes resulting in its transformation to a lymphoblast and culminating in cell division.

E
Annex III

Mandell, Douglas, and Bennett's

Principles and Practice of Infectious Diseases

SIXTH EDITION

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ELSEVIER
CHURCHILL
LIVINGSTONE

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CHAPTER 60

Bronchiolitis

CAROLINE BREESE HALL
JOHN T. McBRIDE

In bronchiolitis we must now contend
with both the disease of the "now" and the "then";
For many such infants a mold has been cast,
perhaps by their unborn and unknown past,
which destines that they shall in time wheeze again.
For them this disease
is the distant, boding knell
of vulnerable lungs
to a microbe's mystic spell.

C.B.H.

Bronchiolitis is an acute viral lower respiratory tract illness that occurs during the first 2 years of life. The illness also has been called "wheezy bronchitis" and "asthmatic bronchitis." Whatever term is applied, the syndrome is caused primarily by viral infections. The characteristic clinical manifestations include an acute onset of wheezing and hyperinflation, most commonly associated with cough, rhinorrhea, tachypnea, and respiratory distress.

The term *bronchiolitis* appears to have been born from a long lineage of confusing sobriquets, including "acute catarrhal bronchitis," "interstitial bronchopneumonia," "spastic bronchopneumonia," "capillary or obstructive bronchiolitis," and "asthmatic bronchiolitis."[1] Bronchiolitis, however, did not become recognized as a distinct entity until the 1940s.[1-5]

ETIOLOGY

Although bronchiolitis was initially thought to be caused by bacteria, viruses and occasionally *Mycoplasma pneumoniae* are now known to be the instigators. Respiratory syncytial virus (RSV) is clearly the major pathogen, and the parainfluenza viruses are the second most commonly isolated agents, with parainfluenza type 3 predominating (Table 60-1 and Fig. 60-1).[6-8] The recently discovered human metapneumovirus also produces bronchiolitis and appears to have clinical and epidemiologic characteristics similar to those of RSV.[9-12] The role of human metapneumovirus in causing respiratory illness in young children awaits further study, but information thus far suggests its contribution may be appreciable but secondary to that of RSV.

A long-term study of respiratory illnesses associated with wheezing in children from a private practice in Chapel Hill, North Carolina,

Agent	Cases (% of Total)	Epidemiologic Occurrence
Respiratory syncytial virus	40-80	Yearly epidemics, winter to spring
Parainfluenza viruses		
Type 3	8-15	Predominantly spring to fall
Type 1	5-12	Epidemics in fall every other year
Type 2	1-5	Fall
Rhinoviruses	3-8	Endemic, all seasons
Adenoviruses	3-10	Endemic, all seasons
Influenza viruses	6-8	Endemic, winter to spring
Mycoplasma pneumoniae	1-7	Endemic, all seasons
Enteroviruses	1-5	Summer to fall
Human metapneumovirus	Unknown	Predominately winter in some areas

showed that RSV, parainfluenza 1 and 3 viruses, adenoviruses, rhinoviruses, and *M. pneumoniae* make up 87% of the isolates obtained from children of all ages.[6] Within the first 2 years of life, RSV accounted for 44% of the isolates, with parainfluenza 1 and 3 viruses and adenoviruses each accounting for about 13%. Similarly, RSV constituted 60% of the isolates obtained from children with bronchiolitis from two group practices in Rochester, New York, over an 11-year period.[13] The second most frequently identified agent was parainfluenza 3 virus, which accounted for 12% of the cases. The relative proportions of these agents may change depending on the population and whether the cases occur as part of an outbreak. However, RSV remains the prime identified cause of bronchiolitis in most ambulatory patients and especially in hospitalized cases, and even in all lower respiratory tract admissions of young infants.[1,4,14-17]

EPIDEMIOLOGY

Bronchiolitis shows a definite seasonal pattern in temperate climates, with a yearly upsurge in the number of cases during winter to early spring.[6,8,13,18] This pattern mirrors that of its prime agent, RSV (see Fig. 60-1). Lesser swells of activity are seen during the fall and spring, when parainfluenza viruses are active.

Bronchiolitis is a common illness during the first year of life, with the peak attack rate generally occurring between 1 and 10 months of age and between 5 weeks and 6 months in hospitalized cases.[5,6,8,14,16,18,19] In outpatients studied in Chapel Hill, the incidence of bronchiolitis was about 11 cases per 100 children for both the first and second 6 months of life.[6,18] In the second year of life, the incidence fell to approximately half that. Among children in the first year of life enrolled in a health maintenance organization in Tucson, Arizona, the rate of occurrence of lower respiratory tract illness was 32.9 cases per 100 children, and 60% of these cases were bronchiolitis.[8]

An appreciable proportion of hospital admissions for infants within the first year of life results from bronchiolitis, especially from RSV. In a review by Breese and colleagues, bronchiolitis was the reason for admission in 4% of their group-practice patients of all ages who required hospitalization for medical illnesses.[19] In a Seattle prepaid medical care group, the rate of hospitalization among infants with bronchiolitis

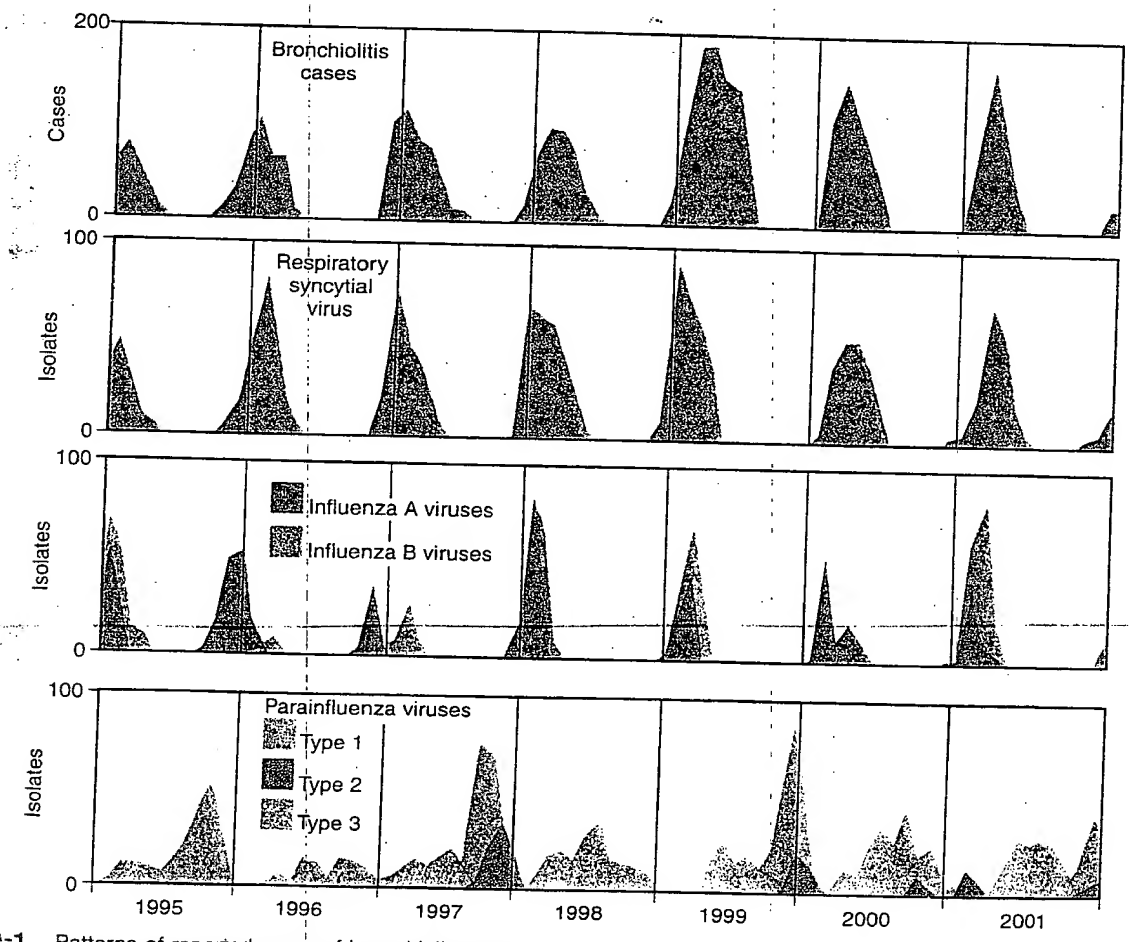

FIGURE 60-1. Patterns of reported cases of bronchiolitis shown in relation to the activity of the major respiratory viruses in Monroe County, New York. Data are obtained from a weekly community surveillance program for infectious disease.

during the first 6 months of life was 6 per 1000 children per year.[5] More recent studies using hospital discharge diagnoses have estimated that RSV caused 50% to 80% of hospitalizations of infants under 1 year of age with bronchiolitis, resulting in 73,400 to 126,300 admissions each year.[16] In this same age group, the RSV hospitalization rate has been estimated to be 25 to 41 per 1000 children.[14,20] RSV hospitalizations for all children under 5 years of age have been judged to be 110,000 each year, resulting in hospital costs of up to $750 million.[21,22] Studies from the Centers for Disease Control and Prevention have estimated the associated mortality for bronchiolitis between 1979 and 1997 was 95 cases (range, 66 to 127) annually for children under 5 years of age, and in infants in the first year of life it was 2.0 per 100,000 live births from 1996 through 1998[23-25]; 55% of these deaths occurred in infants 1 to 3 months of age.

Bronchiolitis is more common in boys, especially among children requiring hospitalization, with a sex ratio of about 1.5 to 1.[6] Other factors described as increasing the chances of hospitalization for bronchiolitis in otherwise normal children include young age, being less than 6 months of age prior to onset of RSV season, young maternal age, lower cord blood antibody titers to RSV, lower socioeconomic status, tobacco smoke exposure, living in crowded surroundings, having older siblings, daycare attendance, lack of breast-feeding, a predisposition to atopy or hyperreactivity of the airway, and illness caused by RSV.[3,6,26-28] Infants at risk for most severe disease, however, are those with underlying conditions, especially cardiopulmonary disease, and preterm gestation.[24] In addition, certain ethnic groups of infants have higher rates of hospitalization for bronchiolitis. Native American and Native Alaskan children have been estimated to have two to three times higher rates than those for all infants in the United States.[25,29]

PATHOPHYSIOLOGY

The term *bronchiolitis* was first used by Engle and Newns in 1940 for the lower respiratory tract disease they observed in young infants that tended to be severe, often fatal, and that was probably viral in origin.[1,2] They carefully described the pathologic findings in these infants dying of bronchiolitis, which over the subsequent half century have been confirmed and expanded.[30]

The pathologic findings in bronchiolitis are characteristically focused on the respiratory epithelium with generalized involvement. The virus initially replicates in the epithelium of the upper respiratory tract, but in the young infant it tends to spread rapidly to the lower tract airways. Early inflammation of the bronchial and bronchiolar epithelium progresses rapidly to necrosis. Subsequently, the epithelium may proliferate and demonstrate cuboidal cells without cilia. Peribronchiolar infiltration, mostly with mononuclear cells, and edema of the submucosa and adventitia occur, and these progress to the observed necrosis and sloughing of the bronchiolar epithelium (Figs. 60-2 and 60-3).

Inflammatory changes of variable severity are observed in most small bronchi and bronchioles. Because resistance to the flow of air is related inversely to the cube of the radius of the airway, the inflammation and edema make the small-lumen airways in infants particularly vulnerable to obstruction. Plugs of necrotic material and fibrin may completely or partially obstruct the small airways.

Smooth muscle constriction does not appear to be important in the obstruction. In areas peripheral to sites of partial obstruction, air becomes trapped by a process similar to a ball-valve mechanism. The negative intrapleural pressure exerted during inspiration allows air to flow beyond the point of partial obstruction. However, on expiration, the size of the lumen decreases with the positive pressure, thereby resulting in increased obstruction and hyperinflation. Thus, although airflow is impeded during both inspiration and expiration, the latter is more affected and prolonged. In areas peripheral to complete obstruction, the trapped air eventually becomes absorbed, which results in multiple areas of atelectasis. This absorptive atelectasis is greatly accelerated when the child is breathing high concentrations of oxygen. The degree of atelectasis or hyperinflation that develops may be greater in infants because collateral channels that maintain alveolar expansion in the presence of airway obstruction are not well developed early in life.

The physiologic correlates of this resistance to airflow are dyspnea, tachypnea, a diminished tidal volume, and a markedly altered distribution of ventilation. In significant areas of lung parenchyma, the ratio of ventilation to perfusion is low, and this produces arterial hypoxemia. When an infant is no longer able to compensate for the disordered gas exchange by increasing ventilation, hypercarbia may ensue. The pathologic process may progress to involve the alveolar walls and spaces, producing an interstitial pneumonitis. Recovery tends to be slow and requires several weeks.

Experimental and clinical studies have suggested that the development of wheezing and the pathogenesis of bronchiolitis in some children, and their risk for subsequent wheezing and pulmonary function abnormalities, are related to the type of inflammatory response initiated by RSV or other viruses and to a predisposition of the host (see Chapter 155). In particular, hypersensitivity responses characterized

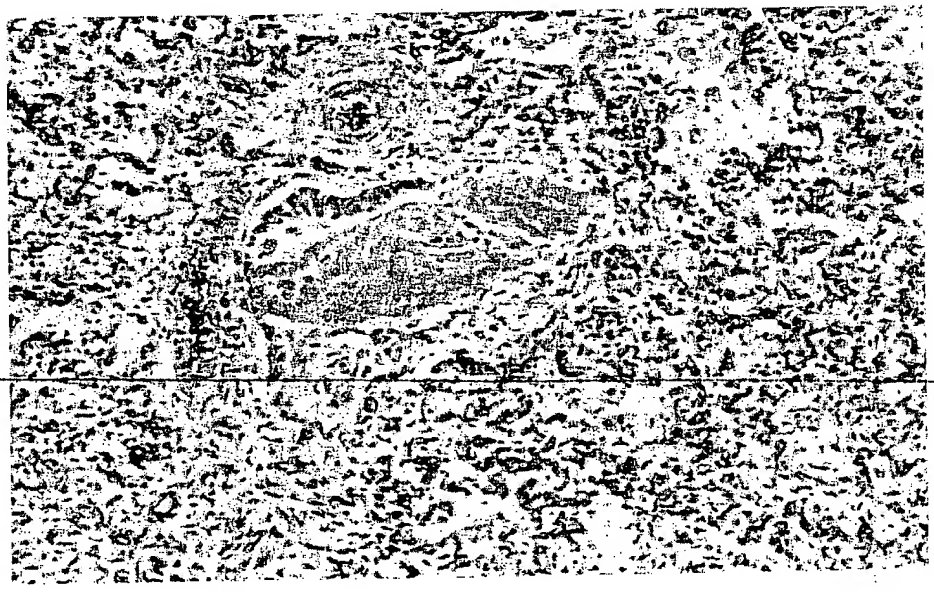

FIGURE 60-2. Inflammation and necrosis in bronchiolitis, resulting in obliteration of the bronchiolar lumen.

by increased levels of virus-specific IgE antibody and certain cellular inflammatory mediators have been related to the development and severity of wheezing in infants and the risk of recurrent wheezing.[31-33] Studies detecting cytokines and chemokines in respiratory secretions of children with bronchiolitis and with recurrent wheezing have suggested the expression of interferon-γ, interleukin (IL)-8, IL-10, and cytokines produced by helper T (Th)1 and Th2 lymphocytes are important in the pathogenesis of bronchiolitis and subsequent recurrent episodes of wheezing.[31-38]

Clarifying the relationship between bronchiolitis and subsequent asthma is complicated by confusion about the pathophysiology of asthma itself.[39] Asthma is a heterogeneous group of disorders engendered by multiple factors, both genetic and environmental. These include not only an atopic predisposition and the environmental risk factors noted earlier but also specific genetic polymorphisms.[3,40-44] Studies on twins have demonstrated that 70% of the risk of developing asthma in early childhood is related to genetic factors,[45] and many of the specific linkages identified to date are related to inflammation. Nevertheless, the disorders in this heterogeneous group share, in various combinations, wheezing, reversible airway obstruction, airway inflammation, and structural airway wall remodeling. Many distinct asthma phenotypes exist, and the following are examples of only three: (1) Some children wheeze repeatedly with respiratory viral infections in the first 5 years of life but have few problems thereafter.[46] (2) Other children with atopy and allergies develop wheezing beyond 5 years of age. Many of these have fewer problems when they become teenagers and adults. (3) Other individuals with adult-onset wheezing are at increased risk of developing irreversible airway obstruction.

Much of asthma, therefore, seems to be related to a dysregulation of airway inflammation. Inherited traits that do not involve the inflammatory response may also be important in some individuals, such as variations in the beta adrenergic receptor or in airway geometry (e.g., size). Any traits that contribute to the dysregulation of airway inflammation or airway dysfunction in asthma may also contribute to the same processes with respiratory viral infections. Thus, the increased incidence of wheezing with subsequent respiratory viral infections among children with a history of bronchiolitis in infancy is not surprising. Nevertheless, the association between bronchiolitis and asthma is not straightforward. Several investigators have demonstrated that children with bronchiolitis in infancy have no increased risk for asthma or abnormal pulmonary function by the time they reach early adolescence.[47]

CLINICAL MANIFESTATIONS

Bronchiolitis may have a variety of appellations, including wheezing, bronchiolitis, infectious asthma, and asthmatic bronchitis, but all refer to the clinical syndrome in young children presenting with wheezing and hyperinflation of the lungs often accompanied by tachypnea. The onset of bronchiolitis, however, consists of upper respiratory tract signs, especially coryza and cough. Commonly, a prodromal period of 1 to 7 days occurs and is marked by fever, which is usually mild, especially with RSV. Sometimes the initial presentation is apnea. Apnea usually appears after 1 to 3 days of upper respiratory signs that are so mild as to go unnoticed and before lower respiratory tract disease is evident. Manifestations of lower respiratory tract involvement become evident after the several days of the prodromal upper respiratory tract signs. The progression of the disease may be reflected initially in the development of a prominent cough, and subsequently by an increase in the respiratory rate and in nonspecific systemic symptoms such as irritability, lethargy, and anorexia. With progression, tachypnea and tachycardia may be marked, although fever may no longer be present. Retractions of the chest wall, flaring of the nasal alae, and grunting are evidence of the increased work of breathing. Cyanosis is rarely observed, even though moderate to severe hypoxemia may be present.[48] Auscultatory findings, which may vary from hour to hour, include wheezing with or without crackles. Increasing dyspnea with decreasing lung sounds on auscultation and diminished movement of air may indicate progressive obstruction and impending respiratory failure.

Dehydration is a common accompaniment of bronchiolitis and results from paroxysms of coughing, which may trigger vomiting, and from a poor oral intake related to the respiratory distress and lethargy. Tachypnea further increases the fluid requirement. Otitis media occurs in 10% to 30% of infants, and mild conjunctivitis and occasionally diarrhea may also be present.

The acute course typically lasts 3 to 7 days. Most infants show improvement within 3 to 4 days and gradually recover over 1 to 2 weeks, but the cough may persist for longer. One study, examining the duration of illness in ambulatory children diagnosed with bronchiolitis, found that the median duration of symptoms was 12 days. After 3 weeks, 18% remained symptomatic, and after 4 weeks, 9% continued to be ill.[49] No factor—sex, age, weight, or respiratory rate—appeared to be predictive of longer illness. The viral etiology was not examined.

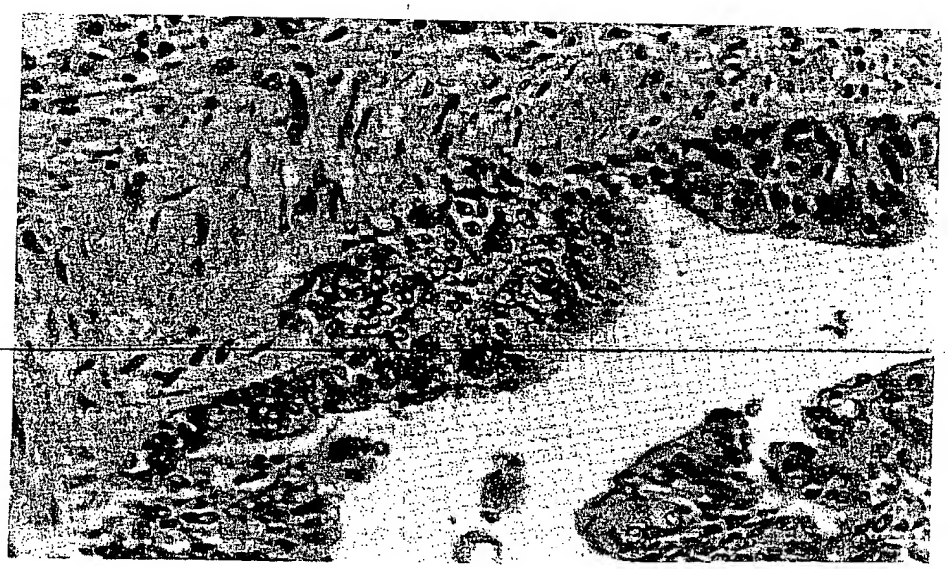

FIGURE 60-3. Inflammation of the bronchiole with regenerating epithelium.

COMPLICATIONS

Almost all children recover from bronchiolitis without difficulty.[3,49] Complications from bronchiolitis are relatively rare, especially in normal children. A number of studies have attempted to predict by epidemiologic and clinical characteristics which children with acute bronchiolitis are most likely to have complicated or severe courses. Infants with underlying diseases, especially cardiac, pulmonary, and immunodeficiency diseases, and those who were premature are most at risk for prolonged or complicated illness.[24,27,50,51] Clinical characteristics at the onset of the acute illness, such as respiratory rate or auscultatory findings, have not been of consistent prognostic value. Diminished arterial oxygen saturation, however, has been associated with complicated illness.[3,50,52] Progression to respiratory failure and prolonged hypoxemia are uncommon with currently available technical and pharmacologic methods of management. If such complications occur, they are most likely to be in infants with compromising underlying conditions and in very young infants.

Cardiovascular abnormalities have been occasionally reported to occur during bronchiolitis in children with no underlying cardiac disease.[53,54] In one study, 2% of infants with bronchiolitis had mild electrocardiographic abnormalities.[54] In a small group of infants with bronchiolitis from RSV or parainfluenza virus, about half were demonstrated to have some transient tricuspid valve regurgitation during the most acute phase of the illness.[53]

Aspiration has also been demonstrated to be a relatively frequent complication in infants with RSV bronchiolitis.[55,56] Infants who received no therapy for aspiration were much more likely to develop reactive airway disease subsequently.

The sequelae of bronchiolitis that occur frequently and are of major concern are recurrent episodes of reactive airway disease, which are accompanied by pulmonary function abnormalities in some children. As discussed previously (see "Pathophysiology"), the link between this and bronchiolitis in infancy is unclear. Nevertheless, the prognosis for most children with subsequent episodes of wheezing during early childhood is good. Some follow-up studies of children who had bronchiolitis diagnosed in infancy have shown that these children at school age had no greater occurrence of reactive airway disease than those without an early history of bronchiolitis.[57,58] The mortality associated with bronchiolitis has been markedly reduced with the advancement in the technology of supportive care. Overall, the mortality in hospitalized infants has been estimated to be less than 1%.[59,60] The mortality rate, however, increases significantly in those children with underlying compromising conditions and is estimated at 3% to 5%.[23,59] In these children, the greatest proportion of bronchiolitis-associated deaths has occurred in infants of low birth weight (<2500 g), especially in those with very low birth weight (<1500 g).[24] Nevertheless, the majority (63%) of all bronchiolitis-associated deaths in children under 1 year of age occurs in those with a normal birth weight.[24]

LABORATORY FINDINGS

The total white blood cell count in children with bronchiolitis is usually within the normal range or slightly elevated.[3,48] In hospitalized infants who are more seriously ill and hypoxemic, the white blood cell count may be elevated, and the differential count may show a leftward shift. Most infants requiring hospitalization will have some degree of hypoxemia on measurement of the arterial oxygen saturation levels. Clinically, this is difficult to assess, because the degree of wheezing and retractions correlates poorly with the level of oxygenation. Only the most severely ill children develop hypercarbia, as most are able to compensate for the compromised gas exchange by increasing the minute ventilation despite the increased work of breathing.[1]

DIAGNOSIS

The diagnosis of bronchiolitis is made most frequently on the basis of the characteristic clinical and epidemiologic findings. However, considerable confusion exists over the exact definition of bronchiolitis.[61] A variety of entities may cause a similar picture of dyspnea and wheezing in the infant. Asthma is not easily differentiated, particularly if it is the infant's first episode. Furthermore, the two diseases may be combined. An appreciable proportion of wheezing episodes occurring in a child with an atopic diathesis may arise from viral infections.[62] RSV in particular has a propensity to induce wheezing in young children. Even in adults with acute RSV infection that is clinically manifested as an upper respiratory tract infection, hyperreactivity of the airways can be detected by pulmonary function testing and may last for 1 or 2 months.[63]

Specific laboratory and radiologic tests are not required for most cases of bronchiolitis.[64] In hospitalized infants, determination of the viral etiology may be helpful for infection control procedures, including cohorting, and when specific antiviral therapy, as for influenza or RSV, is being considered.[51]

Identification of the specific agent of acute bronchiolitis can be made in an appreciable proportion of infants by viral isolation from respiratory secretions, preferably from a nasal wash.[6,65] In most cases, the viruses associated with bronchiolitis may be identified in tissue culture within 3 to 7 days. More commonly used are the rapid viral diagnostic techniques, especially for RSV and influenza A and B viruses, which allow identification of the viral antigen in the respiratory secretions within hours.[66,67] The sensitivity of these assays is variable, and the positive predictive value significantly diminishes when RSV infection or the influenza viruses are not epidemic in the community. The use of culture in addition to the screening rapid antigen detection assay may be of particular benefit when the suspected viral agent is not highly prevalent in the community, and when results of the screening rapid-technique tests are negative. Viral isolation procedures and rapid antigen tests that include multiple viral antigens offer the further advantage of detecting other agents that may be the cause of the illness or that are concurrently present. The sensitivity of detecting the viral etiology appears to be markedly enhanced by the use of reverse transcriptase–polymerase chain reaction (RT-PCR).[67-69] Serologic tests to determine the etiologic agent are rarely helpful in clinical management and may be difficult to interpret, because the young infant has maternally acquired antibody to many of the viral agents of bronchiolitis.

A chest radiograph is not routinely recommended for first-time wheezers in the first year of life if there are no complications or underlying disease.[3] If a chest roentgenogram is obtained, the hallmarks of acute bronchiolitis are hyperinflation with associated depressed diaphragms, hyperlucency of the parenchyma, and decreased costophrenic angles.[70-73] The bronchovascular markings are usually prominent, with linear densities radiating from the hila. Multiple areas of atelectasis of variable degree are also commonly present and difficult to differentiate from the infiltrates of pneumonia. Indeed, both bronchiolitis and pneumonia may be concurrently present, especially with RSV infection.

The abnormalities observed on the chest roentgenogram in acute bronchiolitis often do not correlate with the degree of clinical illness; the child may be severely ill despite minimal findings on the chest roentgenogram. Furthermore, considerable intraobserver and interobserver variation has been observed to occur among radiologists in their assessment of roentgenographic findings for the diagnosis of lower respiratory tract disease in infants, especially those with bronchiolitis.[73]

The differential diagnosis of wheezing in an infant is broad and requires a careful history and examination.[61] Gastric reflux and aspiration may produce a picture that is indistinguishable clinically from acute bronchiolitis. Other considerations include obstruction of the airway by a foreign body, vascular rings, retropharyngeal abscess, and even enlarged adenoids. Wheezing may also be associated with cystic fibrosis, immunodeficiency, and congestive heart failure in young infants.

THERAPY

Supportive care is the mainstay of therapy in both outpatient and inpatient cases. At home, care is aimed primarily at comfort, maintaining adequate hydration, and treating fever if necessary. Antibiotics are

not routinely recommended and should be reserved for cases in which proven coinfection with bacteria exists.[3,64]

Hospitalization is necessary for those infants unable to maintain adequate hydration and for those infants with evidence of increasing lethargy and respiratory distress. This may be signaled by increasing retractions of the chest wall and by tachypnea. However, determination of the respiratory rate in young infants is often confounded by crying and fever, and the normal respiratory rate according to age must be considered.[3]

More than two decades ago, Reynolds and Cook noted for hospitalized infants that "oxygen is vitally important in bronchiolitis, and there is little convincing evidence that any other therapy is consistently or even occasionally useful."[74] Today, the mainstay of therapy for the hospitalized child remains supportive care with oxygen administration to maintain an adequate oxygen saturation level (usually at 92% or greater).[1,3,64,75] Although mist therapy is also commonly employed, its use has not been proven beneficial, and chest physiotherapy has been shown to be of no help.[76,77]

The only specific therapy currently available for hospitalized cases of bronchiolitis caused by RSV is inhaled ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide), a synthetic nucleoside (see Chapter 155). Ribavirin should be considered for therapy only for those infants who have or are at risk of developing severe or complicated RSV infection, such as those with underlying predisposing conditions, especially prematurity and cardiopulmonary disease.[51] The drug is expensive and the relative benefit to cost must be considered on the basis of the individual patient.

Medical therapy commonly includes bronchodilators, corticosteroids, and antibiotics. Evidence for use of any of these has been confusing, contrasting, or incomplete.[64,78] A recent review by the Agency for Healthcare Research and Quality[64] of the efficacy of these therapies in managing bronchiolitis concluded that no single agent could be routinely recommended for the management of bronchiolitis. Convincing evidence for the use of these or other agents was not identified, and some had adverse effects, including budesonide and interferon-alfa-2. The agency's review suggested that several agents should be further studied in correctly designed trials, including nebulized epinephrine, nebulized salbutamol plus ipratropium bromide, nebulized ipratropium bromide, oral or parental corticosteroids (preferably dexamethasone), and inhaled corticosteroids. Despite this lack of evidence of efficacy, these agents, including antibiotics, are used in the majority of infants hospitalized with bronchiolitis and with bronchiolitis identified as caused by RSV.[79,80]

One meta-analysis of bronchodilating agents concluded that no recommendation could be made because determination of their benefit was confounded by the various methods and populations included in the available studies.[81] However, a recent multicenter, randomized, double-blind, controlled study of infants less than 12 months of age with acute bronchiolitis demonstrated that therapy with nebulized epinephrine did not significantly shorten the duration of hospitalization, or the number of days until the infant was ready for discharge.[78] Furthermore, in the cohort of infants who required supplemental oxygen and intravenous fluids, the time until an infant was ready to be discharged was significantly longer for those who received nebulized epinephrine than for those who received placebo. These agents, therefore, are not routinely recommended for infants.[3,64,82] A carefully monitored trial of nebulized bronchodilators in individual cases has been recommended by some. The response should be objectively documented by diminished respiratory distress and improved oxygen saturation. An initial beneficial response, however, may not be seen when bronchodilators are again used later. Repeated use of inhaled bronchodilators in the absence of a positive clinical response is inappropriate.

Studies examining the benefit of corticosteroid therapy have included infants with a clinical diagnosis of bronchiolitis but without a determination of specific viral etiology. The results have been conflicting; most corticosteroids have shown no benefit, and their use is not routinely recommended.[3,64,75] A meta-analysis of therapy with systemic corticosteroids employed in six trials concluded that the duration of hospitalization and symptoms was shortened by 0.43 days.[83] However, two of the six trials included infants with a history of previous wheezing. If these two studies were eliminated from the analysis, the remaining four studies with only first-time wheezers showed that corticosteroids had no significant benefit. Patients with underlying asthma or chronic lung disease (bronchopulmonary dysplasia) who have bronchiolitis related to a lower respiratory viral infection are much more likely to benefit from bronchodilator therapy or a brief course of corticosteroids than are previously well infants. Therefore, such therapy should be considered for infants with bronchiolitis who have bronchopulmonary dysplasia or chronic lung disease, a history of previous wheezing, or a strong family history of asthma.

PREVENTION

Prevention of the clinical entity of bronchiolitis is very difficult because of its multiple etiologies and varying pathogenesis. For bronchiolitis associated with primary RSV infection, antibody preparations, including intravenous immunoglobulin and intravenous immunoglobulin with high titers of neutralizing antibody to RSV (RSV-IVIgG), and more recently monoclonal antibody directed against the F protein of RSV (palivizumab), have been examined for use therapeutically and prophylactically.[51] None of these preparations has shown any benefit in therapy. In high-risk infants, controlled trials have shown a significant reduction in hospitalization for RSV infection when RSV-IVIgG or palivizumab is given on a monthly basis over the 5-month period of RSV activity in the community.[51] Whether infants with high-risk conditions would derive significant benefit compared to the cost remains controversial and in general should be determined on the basis of each infant's circumstances and estimated risk.

BRONCHIOLITIS OBLITERANS

A rare, chronic type of bronchiolitis termed bronchiolitis obliterans has been reported in both adults and children.[84,85] Bronchiolitis obliterans has been cited as an uncommon complication of viral infections, usually viral bronchiolitis, lung transplantation, connective tissue diseases, and inhalation of toxic substances. Often, no cause is identified.[85,86]

In infants, especially those with certain undefined genetic predispositions, the major association has been with adenovirus infection.[87] The disease appears to be particularly prevalent among Native American populations in central Canada and among Polynesians in New Zealand. In some geographic areas, the frequency or clustering of bronchiolitis obliterans cases has been correlated with the occurrence of adenoviral infection in the community.

Pathogenesis

Bronchiolitis obliterans is believed to result from an injury to the bronchioles and smaller airways. The healing process produces large amounts of inflammatory cells, mucoid tissue, granulation tissue, and thickening of the airway walls with connective tissue. This subsequently produces obstruction, bronchiectasis, and even obliteration of the airways.[88,89]

Clinical Findings

The respiratory illness in children initially appears similar to other viral lower respiratory tract illnesses, characterized by cough and lower respiratory tract signs. An interim period of improvement may occur, followed by progressive symptoms of respiratory distress, productive cough, and wheezing. The obstructive respiratory symptoms progress and persist, and the child becomes chronically ill. Many develop bronchiectasis, cor pulmonale, and dependence on oxygen.

Diagnosis

A nodular, diffuse picture, similar to that for miliary tuberculosis, is present on chest roentgenogram. Some patients may also develop Swyer-James syndrome, characterized by a decrease in pulmonary vascular markings and unilateral hyperlucency. Computed tomography reveals bronchiectasis, and bronchography shows that the contrast does not reach the peripheral areas of the lung because of the obstruction.

Annex II Asthma Cardiac asthma

16th Edition

HARRISON'S PRINCIPLES OF Internal Medicine

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water occurs as a consequence of the elevation of systemic venous and capillary pressures and the resultant transudation of fluid into the pulmonary or systemic interstitial space. On the other hand, proponents of the *forward HF* hypothesis maintain that the clinical manifestations of HF result directly from an inadequate discharge of blood into the arterial system. According to this concept, salt and water retention is a consequence of diminished renal perfusion and excessive proximal and distal tubular reabsorption of sodium, the latter through activation of the renin-angiotensin-aldosterone system (RAAS) (Chap. 32).

The rate of onset of HF often influences the clinical manifestations. For example, when a large portion of the left ventricle is suddenly destroyed, as in myocardial infarction, the patient may succumb to acute pulmonary edema, a manifestation of *backward failure*. If the patient survives the acute insult, clinical manifestations resulting from a chronically depressed cardiac output, including the abnormal retention of fluid within the systemic vascular bed, may develop, which is a manifestation of *forward failure*.

SALT AND WATER RETENTION (See also Chap. 32) When the volume of blood pumped by the left ventricle into the systemic vascular bed is reduced, a complex sequence of adjustments occurs that ultimately results in the abnormal accumulation of fluid. This may be considered a two-edged sword. Many of the troubling clinical manifestations of HF, such as pulmonary congestion and edema, are secondary to this excessive retention of fluid (see Fig. 32-1). However, this abnormal fluid accumulation and the expansion of blood volume that accompanies it also constitute an important compensatory mechanism that tends to maintain cardiac output and therefore perfusion of the vital organs. Except in the terminal stages of HF, the ventricle operates on an ascending, albeit depressed and flattened, function curve (see Fig. 215-5), and the augmented ventricular end-diastolic volume characteristic of HF must be regarded as helping to maintain the reduced cardiac output, despite causing pulmonary and/or systemic venous congestion.

CLINICAL MANIFESTATIONS OF HEART FAILURE

RESPIRATORY DISTURBANCES ■ Dyspnea (Chap. 29) In early HF, dyspnea is observed only during exertion, when it may simply represent an aggravation of the breathlessness that occurs normally. As HF advances, dyspnea occurs with progressively less strenuous activity and ultimately it is present even at rest. The principal difference between exertional dyspnea in normal persons and in patients with HF is the degree of exertion necessary to induce this symptom. Cardiac dyspnea is observed most frequently in patients with elevations of pulmonary venous and capillary pressures who have engorged pulmonary vessels and interstitial accumulation of interstitial fluid. The activation of receptors in the lungs results in the rapid, shallow breathing characteristic of cardiac dyspnea. The oxygen cost of breathing is increased by the excessive work of the respiratory muscles required to move air into and out of the congested lungs. This is coupled with the diminished delivery of oxygen to these muscles, a consequence of a reduced cardiac output. This imbalance may contribute to fatigue of the respiratory muscles and the sensation of shortness of breath.

Orthopnea This symptom, i.e., dyspnea in the recumbent position, is usually a later manifestation of HF than exertional dyspnea. Orthopnea results from the redistribution of fluid from the abdomen and lower extremities into the chest during recumbency, which increases the pulmonary capillary pressure, combined with elevation of the diaphragm. Patients with orthopnea must elevate their head on several pillows at night and frequently awaken short of breath and/or coughing if their head slips off the pillows. Orthopnea is usually relieved by sitting upright, and some patients report that they find relief from sitting in front of an open window. In advanced HF, patients cannot lie down at all and must spend the entire night in a sitting position.

Paroxysmal (Nocturnal) Dyspnea This term refers to attacks of severe shortness of breath and coughing that generally occur at night, usually awaken the patient from sleep, and may be quite frightening. Though simple orthopnea may be relieved by sitting upright at the side of the bed with legs dependent, in the patient with paroxysmal nocturnal dyspnea, coughing and wheezing often persist even in this position. Paroxysmal nocturnal dyspnea may be caused in part by the depression of the respiratory center during sleep, which may reduce ventilation sufficiently to lower arterial oxygen tension, particularly in patients with interstitial lung edema and reduced pulmonary compliance. *Cardiac asthma* is closely related to paroxysmal nocturnal dyspnea and nocturnal cough and is characterized by wheezing secondary to bronchospasm—most prominent at night. *Acute pulmonary edema* (Chaps. 29 and 255) is a severe form of cardiac asthma due to marked elevation of pulmonary capillary pressure leading to alveolar edema, associated with extreme shortness of breath, rales over the lung fields, and the expectoration of blood-tinged fluid. If not treated promptly, acute pulmonary edema may be fatal.

Cheyne-Stokes Respiration Also known as *periodic respiration* or *cyclic respiration*, Cheyne-Stokes respiration is characterized by diminished sensitivity of the respiratory center to arterial P_{CO_2}. There is an apneic phase, during which the arterial P_{O_2} falls and the arterial P_{CO_2} rises. These changes in the arterial blood stimulate the depressed respiratory center, resulting in hyperventilation and hypocapnia, followed in turn by recurrence of apnea. Cheyne-Stokes respiration occurs most often in patients with cerebral atherosclerosis and other cerebral lesions, but the prolongation of the circulation time from the lung to the brain that occurs in HF, particularly in patients with hypertension and coronary artery disease, also appears to contribute to this form of disordered breathing.

OTHER SYMPTOMS ■ Fatigue and Weakness These nonspecific but common symptoms of HF are related to the reduction of skeletal muscle perfusion. Exercise capacity is reduced by the limited ability of the failing heart to increase its output and deliver oxygen to the exercising muscles.

Abdominal Symptoms Anorexia and nausea associated with abdominal pain and fullness are frequent complaints and may be related to the congested liver and portal venous system.

Cerebral Symptoms Patients with severe HF, particularly elderly patients with cerebral arteriosclerosis, reduced cerebral perfusion, and arterial hypoxemia, may develop alterations in the mental state characterized by confusion, difficulty in concentration, impairment of memory, headache, insomnia, and anxiety. *Nocturia* is common in HF and may contribute to insomnia.

PHYSICAL FINDINGS (See Chap. 209) In mild or moderately severe HF, the patient appears in no distress at rest except feeling uncomfortable when lying flat for more than a few minutes. In severe HF, the pulse pressure may be diminished, reflecting a reduction in stroke volume, and the diastolic arterial pressure may be elevated as a consequence of generalized vasoconstriction. In severe acute HF, systolic hypotension may be present, with cool, diaphoretic extremities, and Cheyne-Stokes respiration. There may be cyanosis of the lips and nail beds (Chap. 31) and sinus tachycardia. *Systemic venous pressure* is often abnormally elevated, and this may be reflected in distention of the jugular veins. In the early stages of HF, the venous pressure may be normal at rest but may become abnormally elevated, with sustained pressure on the abdomen (positive abdominojugular reflux).

Third and fourth heart sounds are often audible but are not specific for HF, and *pulsus alternans*, i.e., a regular rhythm with alternation of strong and weak cardiac contractions and therefore alternation in the strength of the peripheral pulses, may be present. This sign of severe HF may be detected by sphygmomanometry and in more severe cases even by palpation; it frequently follows an extrasystole and is observed most commonly in patients with cardiomyopathy, hypertensive, or ischemic heart disease.

Pulmonary Rales Moist, inspiratory, crepitant rales and dullness to percussion over the lung bases are common in patients with HF and cl-

Annex I

notomy (the Chamberlain procedure). This approach involves either a right or left parasternal incision and dissection directly down to a mass or node that requires biopsy.

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236 ASTHMA

E. R. McFadden, Jr.

Asthma is defined as a chronic inflammatory disease of airways that is characterized by increased responsiveness of the tracheobronchial tree to a multiplicity of stimuli. It is manifested physiologically by a widespread narrowing of the air passages, which may be relieved spontaneously or as a result of therapy, and clinically by paroxysms of dyspnea, cough, and wheezing. Asthma is an episodic disease, with acute exacerbations interspersed with symptom-free periods. Typically, most attacks are short-lived, lasting minutes to hours, and clinically the patient seems to recover completely after an attack. However, there can be a phase in which the patient experiences some degree of airway obstruction daily. This phase can be mild, with or without superimposed severe episodes, or much more serious, with severe obstruction persisting for days or weeks; the latter condition is known as *status asthmaticus*. In unusual circumstances, acute episodes can cause death.

PREVALENCE AND ETIOLOGY Asthma is a very common disease with immense social impact. The prevalence of asthma is rising in many parts of the world, but it is unclear whether this is due to an actual increase in incidence or merely to the fact that the size of the overall population is growing. It is estimated that 4 to 5% of the population of the United States is affected. Data from the Centers for Disease Control and Prevention suggest that 10 to 11 million persons had acute attacks in 1998, which resulted in 13.9 million outpatient visits, 2 million requests for urgent care, and 423,000 hospitalizations, with a total cost >$6 billion. The impact of the disease appears to fall more heavily on minorities and inner-city African-American and Hispanic persons.

Bronchial asthma occurs at all ages but predominantly in early life. About one-half of cases develop before age 10, and another third occur before age 40. In childhood, there is a 2:1 male/female preponderance, but the sex ratio equalizes by age 30. From an etiologic standpoint, asthma is a heterogeneous disease and genetic (atopic) and environmental factors, such as viruses, occupational exposures, and allergens, contribute to its initiation and continuance.

Atopy is the single largest risk factor for the development of asthma. *Allergic asthma* is often associated with a personal and/or family history of allergic diseases such as rhinitis, urticaria, and eczema; with positive wheal-and-flare skin reactions to intradermal injection of extracts of airborne antigens; with increased levels of IgE in the serum; and/or with a positive response to provocation tests involving the inhalation of specific antigen.

A significant fraction of patients with asthma present with no personal or family history of allergy, with negative skin tests, and with normal serum levels of IgE, and therefore have disease that cannot be classified on the basis of currently defined immunologic mechanisms. These patients are said to have *idiosyncratic asthma* or *nonatopic asthma*. Many patients have disease that does not fit clearly into either of the preceding categories but instead falls into a mixed group with features of each. In general, asthma that has its onset in early life tends to have a strong allergic component, whereas asthma that develops late tends to be nonallergic or to have a mixed etiology.

PATHOGENESIS (See also Chap. 298) Asthma results from a state of persistent subacute inflammation of the airways. Even in asymptomatic patients, the airways can be edematous and infiltrated with eosinophils, neutrophils, and lymphocytes, with or without an increase in the collagen content of the epithelial basement membrane. Overall, there is a generalized increase in cellularity associated with an elevated capillary density. There may also be glandular hypertrophy and denudation of the epithelium. These changes may persist despite treatment and often do not relate to the severity of the disease.

The physiologic and clinical features of asthma derive from an interaction among the resident and infiltrating inflammatory cells in the airway surface epithelium, inflammatory mediators, and cytokines. The cells thought to play important parts in the inflammatory response are mast cells, eosinophils, lymphocytes, and airway epithelial cells. The roles of neutrophils, macrophages, and other cellular constituents of the airways are less well defined. Each of the major cell types can contribute mediators and cytokines to initiate and amplify both acute inflammation and the long-term pathologic changes described (Fig. 236-1). The mediators released produce an intense, immediate inflammatory reaction involving bronchoconstriction, vascular congestion, edema formation, increased mucus production, and impaired mucociliary transport. This intense local event can then be followed by a more chronic one. Other elaborated chemotactic factors (eosinophil and neutrophil chemotactic factors of anaphylaxis and leukotriene B_4) also bring eosinophils, platelets, and polymorphonuclear leukocytes to the site of the reaction. The airway epithelium is both the target of, and a contributor to, the inflammatory cascade. This tissue both amplifies bronchoconstriction and promotes vasodilatation through the release of the compounds shown in Fig. 236-2.

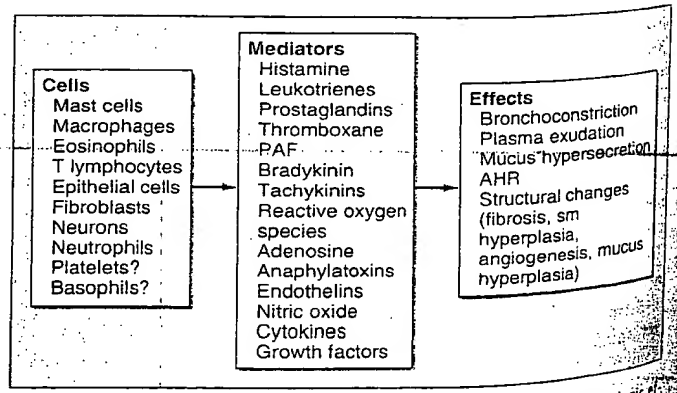

FIGURE 236-1 Cellular sources of inflammatory mediators and their physiologic effects. PAF, platelet-activating factor; AHR, antihyaluronidase reaction. [*From PJ Barnes in E Middleton et al (eds): Allergy Principles and Practice, 5th ed. St. Louis, Mosby, 1998, with permission.*]

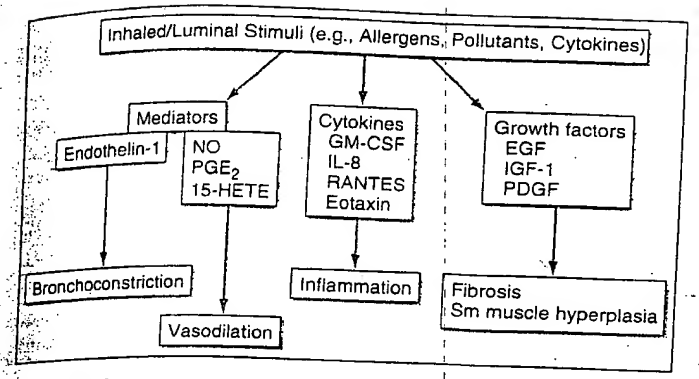

FIGURE 236-2 Inflammatory mediators derived from epithelial sources. NO, nitrous oxide; PGE_2, prostaglandin E_2; GM-CSF, granulocyte-macrophage colony-stimulating factor; IL, interleukin; RANTES, regulated on activation, T cell expressed and secreted; EGF, epidermal growth factor; IGF, insulin-like growth factor; PDGF, platelet-derived growth factor. [*From PJ Barnes, in E Middleton et al (eds): Allergy Principles and Practice, 5th ed. St. Louis, Mosby, 1998, with permission.*]

The eosinophil appears to play an important part in the infiltrative component. Interleukin (IL) 5 stimulates the release of these cells into the circulation and extends their survival. Once activated, these cells are a rich source of leukotrienes, and the granular proteins released (major basic protein and eosinophilic cationic protein) and oxygen-derived free radicals are capable of destroying the airway epithelium, which then is sloughed into the bronchial lumen in the form of Creola bodies. Besides resulting in a loss of barrier and secretory function, such damage elicits the production of chemotactic cytokines, leading to further inflammation. In theory, it can also expose sensory nerve endings, thus initiating neurogenic inflammatory pathways. That, in turn, could convert a primary local event into a generalized reaction via a reflex mechanism. Although an important element in inflammation, the role that the eosinophil plays in establishing and maintaining airway hyperresponsiveness is undergoing reevaluation. Studies using antibodies against IL-5 show a disassociation between the inflammatory and physiologic events following an antigen challenge and blood and sputum eosinophilia. The cytokine network possibly involved in asthma is shown in Fig. 236-3.

T lymphocytes also appear to be important in the inflammatory response. Activated T_H2 cells are present in increased numbers in asthmatic airways and produce cytokines such as IL-4 that initiate humoral (IgE) immune responses. They also elaborate IL-5 with its effect on eosinophils. Data are accumulating that asthma may be related to an imbalance between T_H1 and T_H2 immune responses, but firm conclusions are not yet available.

GENETIC CONSIDERATIONS Although there is little doubt that asthma has a strong familial component, the identification of the genetic mechanisms underlying the illness has proven difficult for such fundamental reasons as a lack of uniform agreement on the definition of the disease, the inability to define a single phenotype, non-Mendelian modes of inheritance, and an incomplete understanding of how environmental factors modify genetic expression. Screening families for candidate genes has identified multiple chromosomal regions that relate to atopy, elevated IgE levels, and airway hyperresponsiveness. Evidence for genetic linkage of high total serum IgE levels and atopy has been observed on chromosomes 5q, 11q, and 12q in a number of populations scattered throughout the world. Regions of the genome demonstrating evidence for linkage to bronchial hyperreactivity also typically show evidence for linkage to elevated total serum IgE levels. Excellent candidate genes exist for specific abnormalities in asthma within the regions that were identified in the linkage studies. For example, chromosome 5q contains cytokine clusters including IL-4, IL-9, and IL-13. Other regions on chromosome 5q also contain the β-adrenergic receptors and the glucocorticoid receptors. Chromosome 12q contains regions that are important in antigen presentation and mediation of the inflammatory response. Chromosome 12q contains two genes that could influence atopy and airway hyperresponsiveness, including nitric oxide synthase.

STIMULI THAT INCITE ASTHMA The stimuli that incite acute episodes of asthma can be grouped into seven major categories: allergenic, pharmacologic, environmental, occupational, infectious, exercise-related, and emotional.

Allergens Allergic asthma is dependent on an IgE response controlled by T and B lymphocytes and activated by the interaction of antigen with mast cell–bound IgE molecules. The airway epithelium and submucosa contain dendritic cells that capture and process antigen. After taking up an immunogen, these cells migrate to the local lymph nodes where they present the material to T cell receptors. In the appropriate genetic setting, the interaction of antigen with a naïve T cell T_H0 in the presence of IL-4 leads to the differentiation of the cell to a T_H2 subset. This process not only helps facilitate the inflammation of asthma but also causes B lymphocytes to switch their antibody production from IgG and IgM to IgE.

Once synthesized and released by B cells, IgE circulates in the blood until it attaches to high-affinity receptors on mast cells and low-affinity receptors on basophils. Most of the allergens that provoke asthma are airborne, and to induce a state of sensitivity they must be reasonably abundant for considerable periods of time. Once sensitization has occurred, however, the patient can exhibit exquisite responsivity, so that minute amounts of the offending agent can produce significant exacerbations of the disease. Immune mechanisms appear to be causally related to the development of asthma in 25 to 35% of all cases and to be contributory in perhaps another third. Higher prev-

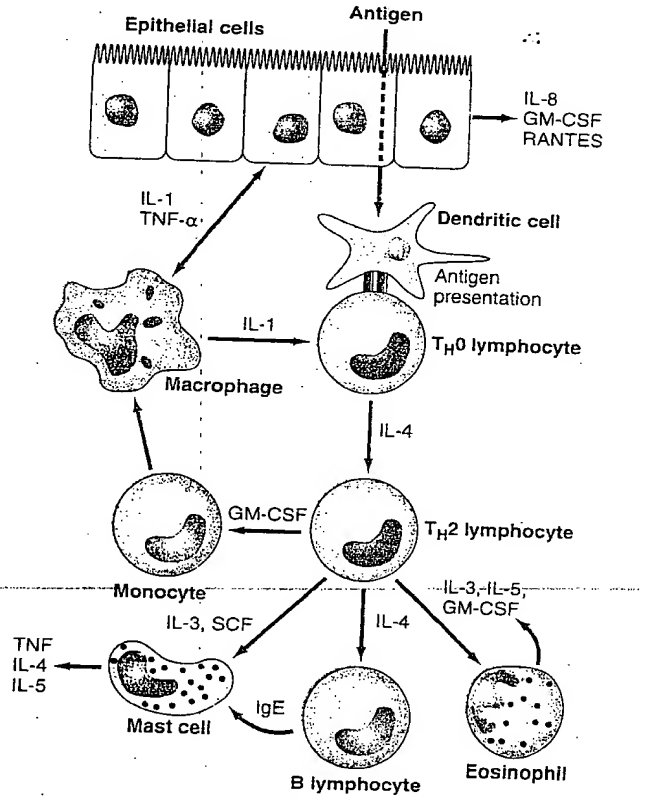

FIGURE 236-3 Cytokine network in allergic asthma. IL, interleukin; GM-CSF, granulocyte-macrophage colony-stimulating factor; RANTES, regulated on activation, T cell expressed and secreted; TNF, tumor necrosis factor; SCS, stem cell factor. [*From PJ Barnes, in E Middleton et al (eds): Allergy Principles and Practice, 5th ed. St. Louis, Mosby, 1998, with permission.*]

alences have been suggested, but it is difficult to know how to interpret the data because of confounding factors. Allergic asthma is frequently seasonal, and it is most often observed in children and young adults. A nonseasonal form may result from allergy to feathers, animal danders, dust mites, molds, and other environmental antigens that are present continuously. Exposure to antigen typically produces an immediate response in which airway obstruction develops in minutes and then resolves. In 30 to 50% of patients, a second wave of bronchoconstriction, the so-called late reaction, develops 6 to 10 h later. In a minority, only a late reaction occurs. It was formerly thought that the late reaction was essential to the development of the increase in airway reactivity that follows antigen exposure. This is now known not to be the case.

The mechanism by which an inhaled allergen provokes an acute episode of asthma depends in part on antigen-antibody interactions on the surface of pulmonary mast cells, with the subsequent generation and release of the mediators of immediate hypersensitivity. Current hypotheses hold that very small antigenic particles penetrate the lung's defenses and come in contact with mast cells that interdigitate with the epithelium at the luminal surface of the central airways. The subsequent elaboration of mediators and cytokines then produces the sequence outlined above.

Pharmacologic Stimuli The drugs most commonly associated with the induction of acute episodes of asthma are aspirin, coloring agents such as tartrazine, β-adrenergic antagonists, and sulfiting agents. It is important to recognize drug-induced bronchial narrowing because its presence is often associated with great morbidity. Furthermore, death sometimes has followed the ingestion of aspirin (or other nonsteroidal anti-inflammatory agents) or β-adrenergic antagonists. The typical aspirin-sensitive respiratory syndrome primarily affects adults, although the condition may occur in childhood. This problem usually begins with perennial vasomotor rhinitis that is followed by a hyperplastic rhinosinusitis with nasal polyps. Progressive asthma then appears. On exposure to even very small quantities of aspirin, affected individuals typically develop ocular and nasal congestion and acute, often severe episodes of airways obstruction.

The prevalence of aspirin sensitivity in patients with asthma varies from study to study, but many authorities feel that 10% is a reasonable figure. There is a great deal of cross-reactivity between aspirin and other nonsteroidal anti-inflammatory compounds that inhibit prostaglandin G/H synthase 1 (cyclooxygenase type 1). Indomethacin, fenoprofen, naproxen, zomepirac sodium, ibuprofen, mefenamic acid, and phenylbutazone are particularly important in this regard. However, acetaminophen, sodium salicylate, choline salicylate, salicylamide, and propoxyphene are well tolerated. The exact frequency of cross-reactivity to tartrazine and other dyes in aspirin-sensitive individuals with asthma is also controversial; again, 10% is the commonly accepted figure. This peculiar complication of aspirin-sensitive asthma is particularly insidious, however, in that tartrazine and other potentially troublesome dyes are widely present in the environment and may be unknowingly ingested by sensitive patients.

Patients with aspirin sensitivity can be desensitized by daily administration of the drug. After this form of therapy, cross-tolerance also develops to other nonsteroidal anti-inflammatory agents. The mechanism by which aspirin and other such drugs produce bronchospasm appears to be a chronic overexcretion of cysteinyl leukotrienes, which activate mast cells. The adverse reaction to aspirin can be inhibited with the use of leukotriene synthesis blockers or receptor antagonists.

β-Adrenergic antagonists regularly obstruct the airways in individuals with asthma as well as in others with heightened airway reactivity and should be avoided by such individuals. Even the selective beta$_1$ agents have this propensity, particularly at higher doses. In fact, the topical use of beta$_1$ blockers in the eye for the treatment of glaucoma has been associated with worsening asthma.

Sulfiting agents, such as potassium metabisulfite, potassium and sodium bisulfite, sodium sulfite, and sulfur dioxide, which are widely used in the food and pharmaceutical industries as sanitizing and preserving agents, can also produce acute airway obstruction in sensitive individuals. Exposure usually follows ingestion of food or beverages containing these compounds, e.g., salads, fresh fruit, potatoes, shellfish, and wine. Exacerbation of asthma has been reported after the use of sulfite-containing topical ophthalmic solutions, intravenous glucocorticoids, and some inhalational bronchodilator solutions. The incidence and mechanism of action of this phenomenon are unknown. When suspected, the diagnosis can be confirmed by either oral or inhalational provocations.

Environment and Air Pollution (See also Chap. 238) Environmental causes of asthma are usually related to climatic conditions that promote the concentration of atmospheric pollutants and antigens. These conditions tend to develop in heavily industrial or densely populated urban areas and are frequently associated with thermal inversions or other situations creating stagnant air masses. In these circumstances, although the general population can develop respiratory symptoms, patients with asthma and other respiratory diseases tend to be more severely affected. The air pollutants known to have this effect are ozone, nitrogen dioxide, and sulfur dioxide. All produce greater effects during periods of high ventilation. In some regions of North America, seasonal concentrations of airborne antigens such as pollen can rise high enough to result in epidemics of asthma admissions to hospitals and an increase in the death rate. These events may be ameliorated by treating patients prophylactically with anti-inflammatory drugs before the allergy season begins.

Occupational Factors (See also Chap. 238) Occupation-related asthma is a significant health problem, and acute and chronic airway obstruction have been reported to follow exposure to a large number of compounds used in many types of industrial processes. In general, the agents can be classified into high-molecular-weight compounds, which are believed to induce asthma through immunologic mechanisms, and low-molecular-weight agents, which serve as haptines or can release bronchoconstrictor substances. High-molecular-weight compounds of importance are *wood and vegetable dusts* (e.g., those of oak, grain, flour, castor bean, green coffee bean, mako, gum acacia, karay, gum, and tragacanth), *pharmaceutical agents* (e.g., antibiotics, piperazine, and cimetidine), *biologic enzymes* (e.g., laundry detergents, pancreatic enzymes, and *Bacillus subtilis*), and *animal and insect dusts, serums, and secretions* (e.g., laboratory animals, chickens, crabs, prawns, oysters, flys, bees, and moths). Troublesome low-molecular-weight compounds are *metal salts* (e.g., platinum, chrome, vanadium, and nickel) and *industrial chemicals and plastics* (e.g., toluene diisocyanate, phthalic acid anhydride, trimellitic anhydride, persulfates, ethylenediamine, *p*-phenylenediamine, western red cedar, azidrocarbonamide, and various dyes). Formaldehyde and urea formaldehyde also fall into this group. It is important to recognize that exposure to sensitizing chemicals, particularly those used in paints, solvents, and plastics, can also occur during leisure or non-work-related activities.

If the occupational agent causes an immediate or dual immunologic reaction, the history is similar to that which occurs with exposure to other antigens. Often, however, patients will give a characteristic cyclic history. They are well when they arrive at work, and symptoms develop toward the end of the shift, progress after the work site is left, and then regress. Absence from work during weekends or vacations brings about remission. Frequently, there are similar symptoms in fellow employees.

Infections Respiratory infections are the most common of the stimuli that evoke acute exacerbations of asthma. Respiratory viruses and not bacteria or allergy to microorganisms are the major etiologic factors. In young children, the most important infectious agents are respiratory syncytial virus and parainfluenza virus. In older children and adults, rhinovirus and influenza virus predominate as pathogens. Simple colonization of the tracheobronchial tree is insufficient to evoke acute episodes of bronchospasm, and attacks of asthma occur only when

symptoms of an ongoing respiratory tract infection are, or have been, present. Viral infections can actively and chronically destabilize asthma, and they are perhaps the only stimuli that can produce constant symptoms for weeks. The mechanism by which viruses induce exacerbations of asthma may be related to the production of T cell–derived cytokines that potentiate the infiltration of inflammatory cells into already susceptible airways.

Exercise Exercise is a very common precipitant of acute episodes of asthma. This stimulus differs from other naturally occurring provocations, such as antigens, viral infections, and air pollutants, in that it does not evoke any long-term sequelae, nor does it increase airway reactivity. Typically the attacks follow exertion and do not occur during it. The critical variables that determine the severity of the postexertional airway obstruction are the levels of ventilation achieved and the temperature and humidity of the inspired air. The higher the ventilation and the lower the heat content of the air, the greater the response. For the same inspired air conditions, running produces a more severe attack of asthma than walking because of its greater ventilatory cost. Conversely, for a given task, the inhalation of cold air markedly enhances the response, while warm, humid air blunts or abolishes it. Consequently, activities such as ice hockey, cross-country skiing, and ice skating (high ventilations of cold air) are more provocative than is swimming in an indoor, heated pool (relatively low ventilation of humid air). The mechanism by which exercise produces obstruction may be related to a thermally produced hyperemia and capillary leakage in the airway wall.

Emotional Stress Psychological factors can worsen or ameliorate asthma. Changes in airway caliber seem to be mediated through modification of vagal efferent activity, but endorphins may also play a role. The extent to which psychological factors participate in the induction and/or continuation of any given acute exacerbation is not established but probably varies from patient to patient and in the same patient from episode to episode.

PATHOLOGY In a patient who has died of acute asthma, the most striking feature of the lungs at necropsy is their gross overdistention and failure to collapse when the pleural cavities are opened. When the lungs are cut, numerous gelatinous plugs of exudate are found in most of the bronchial branches down to the terminal bronchioles. Histologic examination shows hypertrophy of the bronchial smooth muscle, hyperplasia of mucosal and submucosal vessels, mucosal edema, denudation of the surface epithelium, pronounced thickening of the basement membrane, and eosinophilic infiltrates in the bronchial wall. There is an absence of any of the well-recognized forms of destructive emphysema.

PATHOPHYSIOLOGY The pathophysiologic hallmark of asthma is a reduction in airway diameter brought about by contraction of smooth muscle, vascular congestion, edema of the bronchial wall, and thick, tenacious secretions. The net result is an increase in airway resistance, a decrease in forced expiratory volumes and flow rates, hyperinflation of the lungs and thorax, increased work of breathing, alterations in respiratory muscle function, changes in elastic recoil, abnormal distribution of both ventilation and pulmonary blood flow with mismatched ratios, and altered arterial blood gas concentrations. Thus, although asthma is considered to be primarily a disease of airways, virtually all aspects of pulmonary function are compromised during an acute attack. In addition, in very symptomatic patients there frequently is electrocardiographic evidence of right ventricular hypertrophy and pulmonary hypertension. When a patient presents for therapy, the 1-s forced expiratory volume (FEV_1) or peak expiratory flow rate (PEFR) is typically <40% of predicted. In keeping with the alterations in mechanics, the associated air trapping is substantial. In acutely ill patients, residual volume frequently approaches 400% of normal, while functional residual capacity doubles.

Hypoxia is a universal finding during acute exacerbations, but frank ventilatory failure is relatively uncommon, being observed in 10 to 15% of patients presenting for therapy. Most individuals with asthma have hypocapnia and a respiratory alkalosis. In acutely ill patients, the finding of a normal arterial carbon dioxide tension tends to be associated with quite severe levels of obstruction. Consequently, when found in a symptomatic individual, it should be viewed as representing impending respiratory failure, and the patient should be treated accordingly. Equally, the presence of metabolic acidosis in the setting of acute asthma signifies severe obstruction. Cyanosis is a very late sign. Trying to judge the state of an acutely ill patient's ventilatory status on clinical grounds alone can be extremely hazardous, and clinical indicators should not be relied on with any confidence. Therefore, in patients with suspected alveolar hypoventilation, arterial blood gas tensions must be measured.

CLINICAL FEATURES The symptoms of asthma consist of a triad of dyspnea, cough, and wheezing, the last often being regarded as the sine qua non. In its most typical form, all three symptoms coexist. At the onset of an attack, patients experience a sense of constriction in the chest, often with a nonproductive cough. Respiration becomes audibly harsh; wheezing in both phases of respiration becomes prominent; expiration becomes prolonged; and patients frequently have tachypnea, tachycardia, and mild systolic hypertension. The lungs rapidly become overinflated, and the anteroposterior diameter of the thorax increases. If the attack is severe or prolonged, there may be a loss of adventitial breath sounds, and wheezing becomes very high pitched. Furthermore, the accessory muscles become visibly active, and a paradoxical pulse often develops. These two signs are extremely valuable in indicating the severity of the obstruction. In the presence of either, pulmonary function tends to be significantly more impaired than in their absence. It is important to note that the development of a paradoxical pulse depends on the generation of large negative intrathoracic pressures. Thus, if the patient's breathing is shallow, this sign and/or the use of accessory muscles could be absent even though obstruction is quite severe. The other signs and symptoms of asthma only imperfectly reflect the physiologic alterations that are present. Indeed, if the disappearance of subjective complaints or even of wheezing is used as the end point at which therapy for an acute attack is terminated, an enormous reservoir of residual disease will be missed.

The end of an episode is frequently marked by a cough that produces thick, stringy mucus, which often takes the form of casts of the distal airways (Curschmann's spirals) and, when examined microscopically, often shows eosinophils and Charcot-Leyden crystals. In extreme situations, wheezing may lessen markedly or even disappear, cough may become extremely ineffective, and the patient may begin a gasping type of respiratory pattern. These findings imply extensive mucus plugging and impending suffocation. Ventilatory assistance by mechanical means may be required. Atelectasis due to inspissated secretions occasionally occurs with asthmatic attacks. Spontaneous pneumothorax and/or pneumomediastinum occur but are rare.

Less typically, a patient with asthma may complain of intermittent episodes of nonproductive cough or exertional dyspnea. Unlike other individuals with asthma, when these patients are examined during symptomatic periods, they tend to have normal breath sounds but may wheeze after repeated forced exhalations and/or may show ventilatory impairments when tested in the laboratory. In the absence of both these signs, a bronchoprovocation test may be required to make the diagnosis.

DIFFERENTIAL DIAGNOSIS The differentiation of asthma from other diseases associated with dyspnea and wheezing is usually not difficult, particularly if the patient is seen during an acute episode. The physical findings and symptoms listed above and the history of periodic attacks are quite characteristic. A personal or family history of allergic diseases such as eczema, rhinitis, or urticaria is valuable contributory evidence. An extremely common feature of asthma is nocturnal awakening with dyspnea and/or wheezing. In fact, this phenomenon is so prevalent that its absence raises doubt about the diagnosis.

Upper airway obstruction by tumor or *laryngeal edema* can occa-

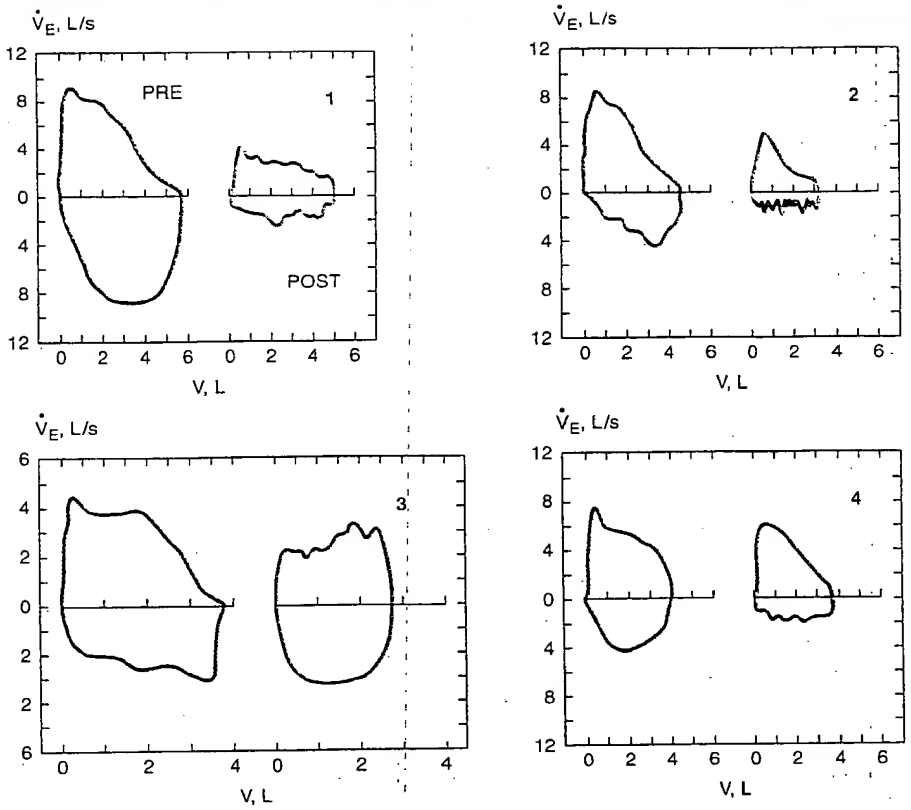

FIGURE 236-4 Representative examples of glottic dysfunction in four patients. The left-hand panels show normal flow-volume curves (red). The right-hand panels (green) represent the development of glottic dysfunction after exercise challenges (green). Note the variable waveforms that can exist. When the patients' attacks ended, the post provocation flow-volume curves returned to normal. $\dot{V}_E$, ventilation; L/s, liters/second; V, L, lung volume in liters.

sionally be confused with asthma. Typically, a patient with such a condition will present with stridor, and the harsh respiratory sounds can be localized to the area of the trachea. Representative flow–volume curves are shown in Fig. 236-4. Diffuse wheezing throughout both lung fields is usually absent. However, differentiation can sometimes be difficult, and indirect laryngoscopy or bronchoscopy may be required. Asthma-like symptoms have been described in patients with glottic dysfunction. These individuals narrow their glottis during inspiration and expiration, producing episodic attacks of severe airway obstruction. Occasionally, carbon dioxide retention develops. However, unlike in asthma, the arterial oxygen tension is well preserved, and the alveolar-arterial gradient for oxygen narrows during the episode, instead of widening as with lower airway obstruction. To establish the diagnosis of glottic dysfunction, the glottis should be examined when the patient is symptomatic. Normal findings at such a time exclude the diagnosis; normal findings during asymptomatic periods do not.

Persistent wheezing localized to one area of the chest in association with paroxysms of coughing indicates *endobronchial disease* such as foreign-body aspiration, a neoplasm, or bronchial stenosis.

The signs and symptoms of *acute left-ventricular failure* occasionally mimic asthma, but the findings of moist basilar rales, gallop rhythms, blood-tinged sputum, and other signs of heart failure (Chap. 216) allow the appropriate diagnosis to be reached.

Recurrent episodes of bronchospasm can occur with *carcinoid tumors* (Chap. 329), *recurrent pulmonary emboli* (Chap. 244), and *chronic bronchitis* (Chap. 242). In chronic bronchitis there are no true symptom-free periods, and one can usually obtain a history of chronic cough and sputum production as a background on which acute attacks of wheezing are superimposed. Recurrent emboli can be very difficult to separate from asthma. Frequently, patients with this condition present with episodes of breathlessness, particularly on exertion, and they sometimes wheeze. Lung scans may not be diagnostic because of the ventilation-perfusion abnormalities characteristic of asthma, and pulmonary angiography may be necessary to establish the correct diagnosis.

Eosinophilic pneumonias (Chap. 237) are often associated with asthmatic symptoms, as are various chemical pneumonias and exposures to insecticides and cholinergic drugs. Bronchospasm is occasionally a manifestation of *systemic vasculitis* with pulmonary involvement.

DIAGNOSIS The diagnosis of asthma is established by demonstrating reversible airway obstruction. *Reversibility* is traditionally defined as a $\geq$15% increase in FEV_1 after two puffs of a β-adrenergic agonist. When the spirometry results are normal at presentation, the diagnosis can be made by showing heightened airway responsiveness to challenges with histamine, methacholine, or isocapnic hyperventilation of cold air. Once the diagnosis is confirmed, the course of the illness and the effectiveness of therapy can be followed by measuring PEFRs at home and/or the FEV_1 in the office or laboratory. Positive wheal-and-flare reactions to skin tests can be demonstrated to various allergens, but such findings do not necessarily correlate with the intrapulmonary events. Sputum and blood eosinophilia and measurement of serum IgE levels are also helpful but are not specific for asthma. Chest roentgenograms showing hyperinflation are also nondiagnostic.

℞ TREATMENT

Elimination of the causative agent(s) from the environment of an allergic individual with asthma is the most successful means available for treating this condition (for details on avoidance, see Chap. 298). Desensitization or immunotherapy with extracts of the suspected allergens has enjoyed widespread favor, but controlled studies are limited and have not proved to be highly effective.

DRUG TREATMENT The available agents for treating asthma can be divided into two general categories: drugs that inhibit smooth-muscle contraction, i.e., the so-called "quick relief medications" (β-adrenergic agonists, methylxanthines, and anticholinergics) and agents that prevent and/or reverse inflammation, i.e., the "long-term control medications" (glucocorticoids, long-acting β_2-agonists, combined medications, mast cell–stabilizing agents, leukotriene modifiers, and methylxanthines (Table 236-1).

Quick Relief Medications ■ *ADRENERGIC STIMULANTS* The drugs in this category consist of the catecholamines, resorcinols, and saligenins. These agents produce airway dilation through stimulation of β-adrenergic receptors and activation of G proteins with the resultant formation of cyclic adenosine monophosphate (AMP). They also decrease release of mediators and improve mucociliary transport. The catecholamines (epinephrine, isoproterenol, and isoetharine) are short-acting (30 to 90 min) and are effective only when administered by inhalational or parenteral routes. Their use has been superceded by the longer acting selective β_2-agonists terbutaline, fenoterol (a resorsinol), and albuterol (a saligenin). The resorsinols and saligenins are highly selective for the respiratory tract and are virtually devoid of significant cardiac effects except at high doses.

Their major side effect is tremor. They are active by all routes of administration and are relatively long-lasting (4 to 6 h). Inhalation is the preferred route because it allows maximal bronchodilation with fewer side effects. In treating episodes of severe asthma, intravenous administration offers no advantages over the inhaled route.

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APPLICATION NUMBER	FILING DATE	GRP ART UNIT	FIL FEE REC'D	ATTY DOCKET NO	DRAWINGS	TOT CLAIMS	IND CLAIMS
09/944,564	09/04/2001	1623	0.00			24	15

CONFIRMATION NO. 8476

FILING RECEIPT

AL-JASSIM Rawaa
24 W. 601 Birdsong Court
Naperville, IL 60540

OC000000006875011

Date Mailed: 10/09/2001

Receipt is acknowledged of this nonprovisional Patent Application. It will be considered in its order and you will be notified as to the results of the examination. Be sure to provide the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION when inquiring about this application. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please write to the Office of Initial Patent Examination's Customer Service Center. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections (if appropriate).**

Applicant(s)

Nida Abdul-Ghani Nassief, Doha, IRAQ;

Domestic Priority data as claimed by applicant

Foreign Applications

UNITED KINGDOM 9904777.1 03/02/1999

If Required, Foreign Filing License Granted 10/06/2001

Projected Publication Date: To Be Determined - pending completion of Missing Parts

Non-Publication Request: No

Early Publication Request: No

**** SMALL ENTITY ****

Title

Asthma/allergy therapy that targets T-lymphocytes and/or eosinophils

Preliminary Class

514

Application/Control Number: 09/944,564
Art Unit: 1623

USPTO
Examiner Patrick T. Lewis, PhD
Fax Number: 001-703-872-9306

Original copy

BY TELEFAX – 5 pages + table of comparison (page 6)+ covering letter and Filing Receipt

August 4, 2004

Response to Detailed Office Action dated May 5, 2004

Dear Examiner,

In response to the above-identified Office Action , please find herewith a response regarding Election/Restriction of the invention. Results of statistical analysis of the clinical trial and annexes will be sent by mail.

Best regards.

Yours Sincerely,

Dr. Nida Nassief

UNITED STATES PATENT AND TRADEMARK OFFICE

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UNITED STATES PATENT AND TRADEMARK OFFICE
WASHINGTON, D.C. 20231
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Naperville, IL 60540

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Early Publication Request: No

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*** SENDEBERICHT ***
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ÜBERTRAGUNG OK

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Application/Control Number: 09/944,564
Art Unit: 1623

USPTO
Examiner Patrick T. Lewis, PhD
Fax Number: 001-703-872-9306

بعثت بالفاكس من المانيا

BY TELEFAX – 5 pages + table of comparison (page 6)+ covering letter and Filing Receipt

August 4, 2004

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N. Nassaim

Dr. Nida Nassief

Office Action Summary	Application No.	Applicant(s)	
	09/944,564	NASSIEF, NIDA ABDUL-GHANI	
	Examiner	Art Unit	
	Patrick T. Lewis	1623	

-- *The MAILING DATE of this communication appears on the cover sheet with the correspondence address* --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION. R-112

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 February 2004*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *25-34* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☐ Claim(s) _____ is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *25-34* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

Response to Office Action dated May 5, 2004.

Applicant's Response dated February 11, 2004

Page 2 of the Office Action:

No response.

Election/Restriction

Page 3 of the Office Action:

Invention 1 as identified by the examiner in the Detailed Office Action " a pharmaceutical composition consisting essentially of glycophosphopeptical and a method of treatment of allergy and asthma" **have been elected.**. This invention includes claims 25-27 inclusive.

The non-elected claims will be withdrawn. According to the Office Action, the other inventions described by the Examiner might be rejoined or I will have the right to file a Divisional Application.

Similarity of invention I and invention II

The relationship between the two active agents Glycophosphopeptical and Nigella sativa is based on disclosed commonality of the mode of operation, function and effect, rather than similarity of the active agent.

The invention as claimed has been limited to the use of the Th1 stimulating agents glycophosphopeptical and pure seeds of Nigella sativa in the manufacture of medicaments for the treatment and/or prophylaxis of asthma/allergy. They have similar therapeutic properties, utility of such

properties and uniqueness of the selected clinical and laboratory variables that were used to assess improvement after a course of treatment.

The nature and significance of the differences between the prior art and the claimed invention as clear from the table of comparison between glycophosphopeptical or/and Nigella sativa asthma medication and current asthma preventive therapy. It is very clear that the claimed subject matter will function in an equivalent manner (last page of the report.

Following are more detailed description of the totality of evidence in relation to the uniqueness of the invention and the mutually exclusive characteristics of the inventive approach for the treatment and/or prophylaxis of asthma/allergy with the two Th1 stimulating agents (glycophosphopeptical or seeds of Nigella sativa) that was perceived through the following observations:

1. The same unique onset of action, magnitude and pattern of changes in clinical assessment criteria
   during treatment with both agents.

2. The similarity in the laboratory main outcome of the clinical trials, in particular sputum eosinophils that are considered as the pharmacological target site.

3. A short-course 5-days treatment using either of the two agents resulted in the same unique long-
   term clinical remission term.

4. The similarity in the dosage and duration of the treatment that was effective to:

- Switch-off the airway eosinophilic inflammation.
- Reduce mucus secretion and as a mucolytic agent.
- Reduce symptom scores significantly.
- Restore airways patency as measured by a Pulmonary Function Test.

Such treatment is unknown and totally unexpected from the prior art. Statistical analysis of results of clinical trial will be sent by mail.

5. The most important and unique achievement is a corresponding permanent effect both for glycophosphopeptical and Nigella sativa has been experimentally verified, in respect of the treatment of allergic rhinosinusitis, by means of X-ray photographs of the paranasal sinuses of patients subjected to corresponding treatments.

Annex I shows copies of two X-ray photographs, the top one showing the paranasal sinuses of a patient suffering from allergic rhino-sinusitis who had previously undergone conventional therapy, but before the inventive treatment with glycophosphopeptical, and the bottom one is a corresponding X-ray after the inventive treatment. From the bottom photo it can be seen that the treatment led to very good resolution of the mucosal thickening of the right maxillary antrum, better aeration of the nasal cavity and mild-moderate resolution of the turbinate hypertrpophy.

Annex II shows copies of two X-ray photographs, the top one showing the paranasal sinuses of a patient suffering from chronic allergic rhinosinusitis and asthma who had previously undergone conventional therapy, but before the inventive treatment with Nigella sativa, and the bottom one is a corresponding X-ray after the inventive treatment. From the bottom photo it can be seen that the treatment led to good resolution of the mucosal thickening of the right and left maxillary antrum, better aeration of the nasal cavity and good resolution of the turbinate hypertrpophy

Annex III is a copy of a review article, published in 2003, regarding the treatment of allergic rhinitis by intranasal steroid sprays, which is regarded as the best conventional treatment. This article mentions only an improvement in patient symptoms. These conventional treatments do not lead to resolution of the mucosal thickening, as is produced by the inventive treatments, and seen in Annex I and Annex II, bottom photographs.

The inventive treatments with both glycophosphopetical and Nigella sativa are hence characterized by a common effect differentiating them from conventional treatments.

Newly-introduced Annex III attests that the best known treatment with corticosteroids merely provide symptomatic relief.

Unity of Invention

Each of the aspects of the invention – Th1 stimulating agent selected from glycophosphopeptical and Nigella sativa seeds – thus provides a common contribution over the state of the art, when considered in the overall context of the claims.

In actual fact, the development of the whole invention and the linked hypothesis occurred as a single jigsaw puzzle, the different parts were placed one-by-one to form a masterpiece that resolves an international enigma! Without the guiding lights of each step it is rather impossible to complete the whole masterpiece. This is the factor underlies the success of my invention to produce a successful treatment for a chronic disabling disease (as considered by the FDA) when others fail; I find it rather difficult to cut it into pieces and bits!

This **common contribution** could be expressed in an additional claim :

> Use of a Th1 stimulating agent selected from glycophosphopeptical and pure seeds of Nigella sativa in the manufacture of a medicament for the treatment and/or prophylaxis of asthma/allergy in a mammal such as a human, wherein the medicament is presented in a form for short term therapy by administration over a period 3 to 30 days, preferably over 5 days, to produce a long term clinical remission over a period of months.

Similarity of invention II and invention III, IV and V

Invention II and III are related as a product and process of use. Both are classified in the same class 424 in the Office Action. The process is not more

than a technique that that was used to define the invention.. In this product claim I have used a novel process as one of the criteria of the invention and one design for its use; the novel nonobvious product was used to obtain a novel end result.

If the Examiner will accept my argument above then the different uses of the allowed product claim will be rejoined in relation to invention III, IV and V in relation to invention II

The end of the report

9 x 8

Table: Comparison between NidAsthma (Glycophosphopeptical) asthma medication and current preventive asthma therapy that brings effective control of symptoms and spares oral steroid use

Drug	Duration of treatment	Onset of clinical response	% decrease in symptom score	Decrease in sputum eosinophils	Lowering of total serum IgE	Changes in mucus	Type of asthma	Prolonged effect
NidAsthma Nigella sativa (Glycophosphopeptical)	5 DAYS ONLY Taken Orally	DAY 3	65-100%	70-90%	50% within 8 weeks	Decreased quantity and viscoelasticity	Intrinsic & allergic	YES
Th2 inhibitor (Ref 1)	8 weeks or more oral	Week 3 &4	40%	----	Significant from baseline at week 4	----	Severe steroid dependancy	NO
Monoclonal anti-IgE (Ref 2 & 3)	21 weeks or more IV injection	Week 12	30% in daytime and night time symptom score (main outcome)	Increase following allergen challenge was attenuated	Within one hour day 0 (Immune complex). Return to base line within 4-5 half-lives of the drug.	----	Allergic only	NO
Cromolyn	Continuos 2 spincaps QDS			Significant decrease in the responder group	Has been shown to reduce IgE synthesis (Ref 6).	----	Allergic only Mostly children	NO
Anti-leukotrienes (Ref 4)	Continuos oral	Occurs within one day	Reduction from baseline in day- time asthma symptom score (-23%), night time awakening (-19%)	Peripheral blood eosinophils: Significant reduction over the 13-week treatment period		----		NO
Inhaled Corticosteroids Currently the 1st Line treatment Used widely for the past 10 years	Continuous for YEARS Daily multiple inhalations Many preparations available	Within 2 weeks	Currently most effective. Results according to prep used. 53% inc. in asthma control days, & 22% in nights without awakening	Peripheral blood eosinophils: 19% change from baseline	Systemic corticosteroids ENHANCE IgE synthesis, raising the concern that early administration of corticosteroids may enhance the development of atopy and asthma (ref 5)	Four weeks treatment with low-dose beclomethasone dipropionate was NOT associated with large reductions in markers of eosinophilic inflammation, broncho-vascular permeability, or mucus hypersecretion (Ref 5).	Intrinsic & allergic	NO
Immunotherapy	Optimal duration unknown, 3-5 years for patients who have had a good therapeutic response Weekly injection Serious side effects		The combined odds of symptomatic improvement was 3-2 (95% CI 2.2-4.9) Using mixture of allergen in unselected asthma showed NO significant difference from placebo (Ref 7).	Inhibit immediate release of mast cell mediators and eosinophil number in nasal lavage in response to allergen provocation.	Serum allergen-specific IgE concentrations initially rise and then gradually fall to baseline levels over months, total serum IgE increase. Oligonucleotide therapy leads to a reduced specific IgE		Allergic only	YES 3-5 years therapy resulted in prolonged effect up to 3 years

Ref 1: Tamaoki J., et al. Effect of suplatast tosilate, a Th2 cytokine inhibitor, on steroid-dependant asthma. The Lancet 2000, July 22 ; 536: 273-8.
Ref 2 : Milgrom H., et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. The New Eng J of Med 1999, Dec 23; 341(26): 1966-1973.
Ref 3: Fahy J. V. Reducing IgE levels as a strategy for the treatment of asthma. Clin Exp Allergy 2000; 30 (Suppl 1): 16-21.
Ref 4: Robert a., et al. Zafirlukast improves asthma symptoms and quality of life in patients with moderate reversible airway flow obstruction. J Allergy Clin Immunol 1998 Dec: 102 (6 part 1): 935-941.
Ref 5: Fahy-JV, Boushey-HA. Effect of low-dose beclomethasone dipropionate on asthma control and airway inflammation. Eur-Respir-J 1998 June; 11 (6); 1240-7.
Ref 6: Peter Van Asperen. Can asthma be prevented? Emirate Medical Journal 1999; 17 (2): 107-108.
Ref 7: WHO position paper, Allergen Immunotherapy: therapeutic vaccines for allergic diseases. Allergy 1998; 53 (44 suppl) :17.

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Nigella sativa and

Table: Comparison between ~~NidAsthma~~ (Glycophosphopeptical) asthma medication and current preventive asthma therapy that brings effective control of symptoms and spares oral steroid use

Drug	Duration of treatment	Onset of clinical response	% decrease in symptom score	Decrease in sputum eosinophils	Lowering of total serum IgE	Changes in mucus	Type of asthma	Prolonged effect
~~NidAsthma~~ Nigella sativa (Glycophosphopeptical)	**5 DAYS ONLY Taken Orally**	**DAY 3**	**65-100%**	**70-90%**	**50% within 8 weeks**	**Decreased quantity and viscoelasticity**	**Intrinsic & allergic**	**YES**
Th2 inhibitor (Ref 1)	8 weeks or more oral	Week 3 &4	40%	----	Significant from baseline at week 4	----	Severe steroid dependancy	**NO**
Monoclonal anti-IgE (Ref 2 & 3)	21 weeks or more IV injection	Week 12	30% in daytime and night time symptom score **(main outcome)**	Increase following allergen challenge was attenuated	Within one hour day 0 **(immuno complex).** Return to base line within 4-5 half-lives of the drug.	----	Allergic only	**NO**
Cromolyn	Continuous 2 spincaps QDS			Significant decrease in the responder group	Has been shown to reduce IgE synthesis **(Ref 6).**	----	Allergic only Mostly children	**NO**
Anti-leukotrienes (Ref 4)	Continuos oral	Occurs within one day	Reduction from baseline in day- time asthma symptom score (-23%), night time awakening (-19%)	**Peripheral blood** eosinophils: Significant reduction over the 13-week treatment period		----		**NO**
Inhaled Corticosteroids Currently the 1st Line treatment Used widely for the past 10 years	**Continuous for YEARS** **Daily multiple inhalations** Many preparations available	Within 2 weeks	Currently most effective. Results according to prep used. 53% inc. in asthma control days, & 22% in nights without awakening	**Peripheral blood** eosinophils: 19% change from baseline	**Systemic** corticosteroids ENHANCE IgE synthesis, raising the concern that early administration of corticosteroids may enhance the development of atopy and asthma **(ref 5)**	**Four weeks** treatment with low-dose beclomethasone dipropionate was **NOT** associated with large reductions in markers of eosinophilic inflammation, broncho-vascular permeability, or mucus hypersecretion (Ref 5).	Intrinsic & allergic	**NO**
Immunotherapy	Optimal duration unknown, **3-5 years** for patients who have had a good therapeutic response Weekly injection **Serious side effects**		The combined odds of symptomatic improvement was **3-2** (95% CI 2.2-4.9) Using mixture of allergen in unselected asthma showed NO significant difference from placebo **(Ref 7).**	Inhibit immediate release of mast cell mediators and eosinophil number in nasal lavage in response to allergen provocation.	Serum allergen-specific IgE concentrations initially rise and then gradually fall to baseline levels over months, total serum IgE increase. **Oligonucleotide therapy** leads to a reduced specific IgE		Allergic only	**YES** **3-5 years therapy** resulted in prolonged effect up to 3 years

Ref 1: Tamaoki J., et al. Effect of suplatast tosilate, a Th2 cytokine inhibitor, on steroid-dependant asthma. The Lancet 2000, July 22 ; 536: 273-8.
Ref 2 : Milgrom H., et al. Treatment of allergic asthma with monoclonal anti-IgE antibody. The New Eng J of Med 1999, Dec 23; 341(26): 1966-1973.
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Ref 6: Peter Van Asperen. Can asthma be prevented? Emirate Medical Journal 1999; 17 (2): 107-108.
Ref 7: WHO position paper, Allergen Immunotherapy: therapeutic vaccines for allergic diseases. Allergy 1998; 53 (44 suppl) :17.

Application/Control Number: 09/944,564
Art Unit: 1623

USPTO
Examiner Patrick T. Lewis, PhD
Fax Number: 001-703-872-9306

BY TELEFAX – 5 pages + table of comparison (page 6)+ covering letter and Filing Receipt

August 4, 2004

Response to Detailed Office Action dated May 5, 2004

Dear Examiner,

In response to the above-identified Office Action , please find herewith a response regarding Election/Restriction of the invention. Results of statistical analysis of the clinical trial and annexes will be sent by mail.

Best regards.

Yours Sincerely,

N. Nassim

Dr. Nida Nassief

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Results of clinical study of the efficacy of glycophosphopeptical and *N sativa* in asthma patients

Please refer from page 13 line 16 to page 14 line 4 of the application.

A comparison of the results from the clinical studies of glycophosphopeptical or *Nigella sativa* extract are shown in figure 1 and table 1 . There appears to be a placebo effect for the first few days. However by day 7 both *Nigella sativa* extract and glycophosphopeptical patients showed a highly statistically significant improvement in asthma symptom scores compared with placebo (P>0.001), and this was sustained at days 14 and 21. **The effects of glycophosphopeptical and Nigella sativa are rather identical!**

Figure 1: Comparison of the effects of glycophosphopeptical and *Nigella sativa* on asthma composite symptom scores

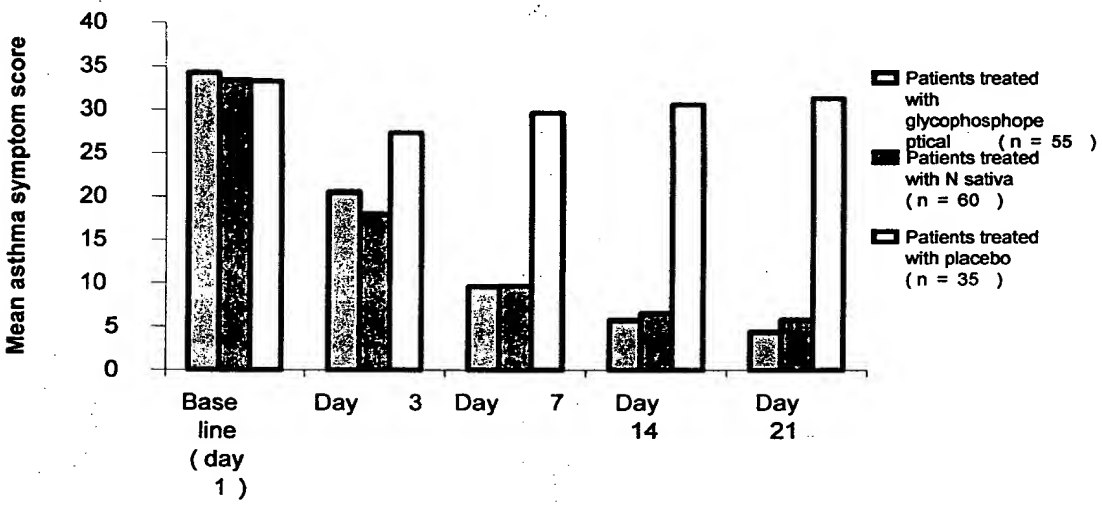

Table 1: Comparison of the effects of glycophosphopeptical and *Nigella sativa* on asthma composite symptom scores

Asthma symptom scores	Patients treated with glycophosphopepti	Patients treated with *N sativa*	Patients treated with placebo	Statistical significance **

Mean +/- SEM	cal (n=55)	(n=60)	(n=35)	
Base line (day 1)	34.21 ± 0.42 * †	33.39 ± 0.3 †	33.2 ± 0.52*	* P<0.01 †P0.01
Day 3	20.54 ± 0.71	17.99 ± 1.2	27.3 ±1.19	Not significant
Day 7	9.65 ± 0.99 * †	9.71 ± 0.94 *	29.7 0.99 †	* P<0.01 †P0.01
Day 14	5.8 ± 0.84 * †	6.55 ± 0.7 *	30.62 ± 0.91 †	* P<0.01 †P0.01
Day 21	4.38 ± 0.9 * †	5.82 ± 0.71 *	31.43 ± 0.66 †	* P<0.01 †P0.01

Results of clinical study of the efficacy of glycophosphopeptical and *N sativa* And comparison to conventional therapy in allergic rhinitis

Please refer to page 12 line 9 of the application for the symptom score of rhinitis.

I will summaries the primary outcome of conventional therapy on rhinitis, which is symptomatic improvement. In comparison, I will submit bar chart that show results of clinical trial treating patients with allergic rhinitis using glycophosphopeptical and Nigella sativa. Striking feature is that both produce the same clinical outcome. The improvement in the sense of smell, as one parameter in improvement of "Quality Of Life", is unprecedented. In addition I will provide X-ray films showing the unique feature of this treatment on resolution of the chronic inflammatory changes from using my invention.

Current allergic rhinitis medications

Allergic rhinitis, a major airway disease that is a risk factor for asthma, warrants extended diagnostic procedures and well-tolerated therapy that encompasses the entire airway, addresses multiple steps in allergic inflammatory cascade, and is effective on nasal, ocular, dermal, and asthma total symptoms (Ref All 1, 2002, abstract, last paragraph).

For many years, oral antihistamines have been first-line medication in the treatment of allergic hypersensitivity reactions including allergic rhinitis, rhinoconjunctivits and urticaria, antihistamines are decongestants. The second effective treatment is intranasal glucocorticoids.

In order to impress the Examiner with the superiority of my invention and the results obtained from my clinical trial. in the following 2 pages I will try to tabulate the primary efficacy assessment and main outcome of clinical trials of conventional treatment of rhinitis. **The references used are from Allergy supplements that were issued specifically for that purpose, one of them is Expanding the Anti-Allergy Horizon 2002.**

The primary efficacy assessment was the mean change from baseline in the total symptom score. In Medline search I was unable to find any reference for changes in the X-ray findings following treatment with current medications both antihistamines or intranasal glucocorticoids. As desloratidine is a new approach in the treatment of allergy that uniquely attenuates patient ratings of nasal congestion (Ref All 2, page 14, abstract). I am referring to multicentre, randomized, double-blind, placebo controlled investigations using deslaoratidine. It is selective H1-receptor antagonist.

The last row in the table will be related to intranasal glucocorticoids. In addition I will describe the symptom scores that are considered as primary outcome!

The scores used in all the clinical trials are:
First, nasal symptoms of itching, stuffiness/congestion, rhinorhoea, and sneezing. (Ref All 1, page 16, column 2 and Ref All 2, page 32, ,column 2).
Second, non-nasal symptoms(itching or burning eyes, itching of ears or palate, eye redness, and tearing) (Ref 1, page 16, column 2 and Ref 2, page 32 ,column 2).
Third, efficacy against multiple disease manifestations of allergy: means that it is effective in the treatment of both seasonal allergic rhinitis and other allergic diseases.
Fourth, dermal symptoms: itching, hives/pustules, dryness (Ref 1, page 16, column 2) and number of hives, size of the largest hive Ref 1, page 15, column 2, second paragraph). .
The fifth goal of treatment should be the improvement of quality of life measures in chronic idiopathic urticaria: Given the burden of allergic diseases on psycho-social well being, i. e.

impaired sleep and impaired ability to conduct daily activities (Ref 1, page 17, figure 2 and Ref 2, page 34 , column 2).

Table 1: Primary efficacy assessment and outcome of antihistamine treatment of allergy using desloratadin.

Clinical trial	Patient No.	Primary outcome
A- Seasonal allergic rhinitis treated desloratadine 5 mg once daily. Multi centre, randomized, double-blind, placebo controlled investigations	172 during spring allergy season	The primary efficacy assessment was the mean change from baseline in the total symptom score averaged over the 2-week study period: 28%. Reduction produced by placebo 13%, P<0.01
B- Seasonal allergic rhinitis treated desloratadine 5 mg once daily. Multi centre, randomized, double-blind, placebo controlled investigations	164 during autum allergy season	The primary efficacy assessment was the mean change from baseline in the total symptom score averaged over the 2-week study period: 30%. Reduction produced by placebo 22%%, P=0.02 Ref 61
C- Perennial allergic rhinitis treated desloratadine 5 mg once daily.		The primary efficacy assessment was the mean change from baseline in the total symptom score averaged over the 4-week study period, P=0.005 Ref 62
D- Seasonal allergic rhinitis + asthma 2 clinical trials, mild-to-moderate asthma worsened during autumn /spring allergy season treated desloratadine 5 mg once daily	613	Primary outcome continue
E- Seasonal allergic rhinitis treated with budesonide nasal spray twice daily treatment for 5	121	Primary outcome was the change in combined nasal symptom scores. Quality of life measured in 121 patients by means

weeks		of Rhinocnjunctivitis Quality of Life Questionnaire: greater than fluticasone. Improvement in scores were significantly superior to placebo
E- Seasonal allergic rhinitis treated with fluticasone		Same criteria were used in assessment

The reference in the table are:

A- (Ref All 2, page 32 , column 2)

B- (Ref All 2, page 32 , column 2)

C- (Ref All 2, page 34 , column 2)

D- (Ref All 2, page 32 , column 2, last paragraph = page 33)

E- (Ref All 4,Abstract and conclusion).

Posterior rhinometry was measured in a clinical trial (Ref All 5, figure 2, page 42).

New therapies should intervenes in the systemic allergy inflammatory cascade and provide clinical efficacy that extends to multiple allergic disease state. In addition, these new therapies should present no additional safety issues, offer improvements over existing therapies, and have an impact on disease-impaired quality of life.

Efficacy against multiple disease manifestations of allergy means that it is capable of treating both seasonal and perennial allergic rhinitis, patients with seasonal allergic rhinitis and asthma it relieves asthma symptoms and decrease the use of rescue medication. Also relieves the symptoms of chronic idiopathic urticaria.

References:

All 1. C. Bachert. Therapeutic points of intervention and clinical implications: role of desloratidine. Allergy57, 2002: suppl. 75;13-18.

All 2. C. Bachert. Decongestant efficacy of desloratadine in patients with seasonal allergic rhinitis. Allergy 2001:56; 14-20.

All 3. P. Van Cauwenberge Advances in allergy management Allergy Supplement 75 Vol 57 2002; 29 – 35.

All 4. G. Ciprandi, et al. Effects of budesonide and fluticasone propionate in a placebo-controlled study on symptoms and quality of life in seasonal allergic rhinitis.

All 5. N. Frossard et al. Comparing the H1 profile of second generation antihistamines. Allergy 2000. 55; 40-45.

Clinical study of the efficacy of glycophosphopeptical and *N sativa* in other allergies

The results of clinical trial using the scoring system in page 12 lines 10, 11 and 13 of the application were similar foe glycophosphopeptical and Nigella sativa.

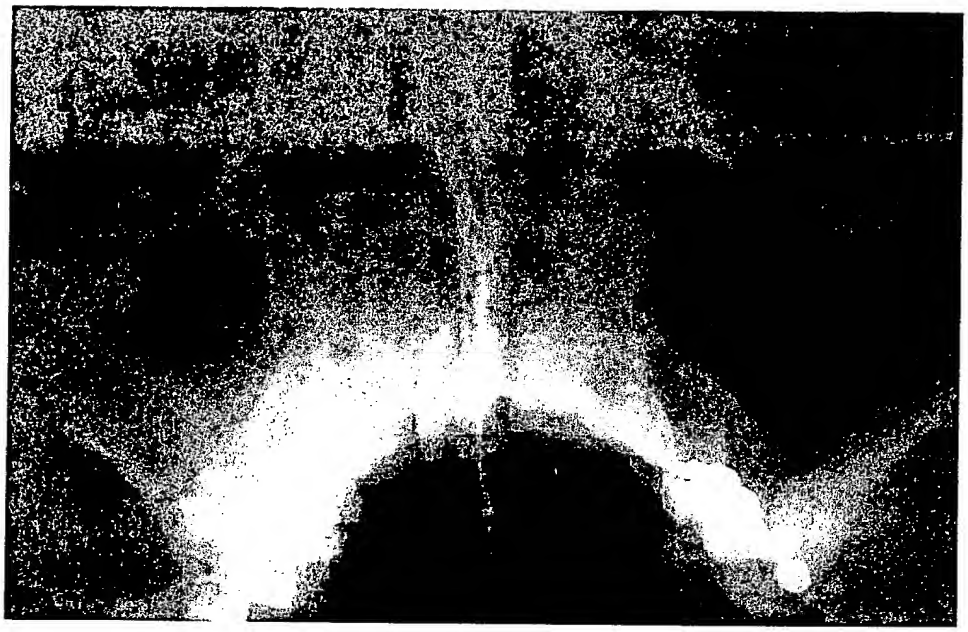

Figure 1: Photographs of X-ray Paranasal sinuses (PNS) of a 42 years old male patient, with a 15 years history of allergic rhino-sinusitis. Figure 1: X-ray PNS shows thickening of the mucosal lining of the right maxillary antrum and bilateral hypertrophy of nasal turbinates, patient treated by conventional therapy.

Figure 2: same patient, 5 months after treatment with 2 courses of immunoferon therapy, showing very good resolution of the mucosal thickening of the right maxillary antrum, better aeration of the nasal cavity and mild-moderate resolution of the turbinate hypertrophy.

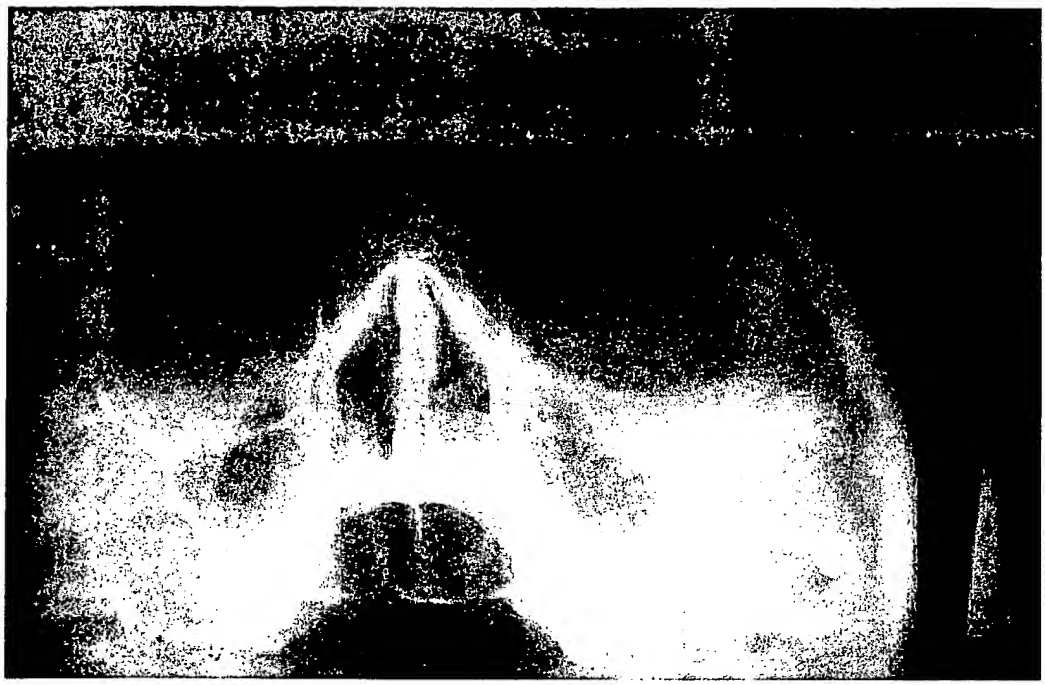

Figure 3: Photographs of X-ray Paranasal sinuses (PNS) of a 37 years old female patoent, with chronic allergic rhinosinusitis and asthma. Figure 3: X-ray PNS shows veiling and thickening of the mucosal lining of the both maxillary antrum and bilateral hypertrophy of nasal turbinates, patient treated by conventional therapy.

Figure 4: same patient in fig. 3, 4 months after treatment with one courses of Nigella sativa therapy, showing good resolution of the mucosal thickening of the both maxillary antrum, better aeration of the nasal cavity and good resolution of the turbinate hypertrophy.

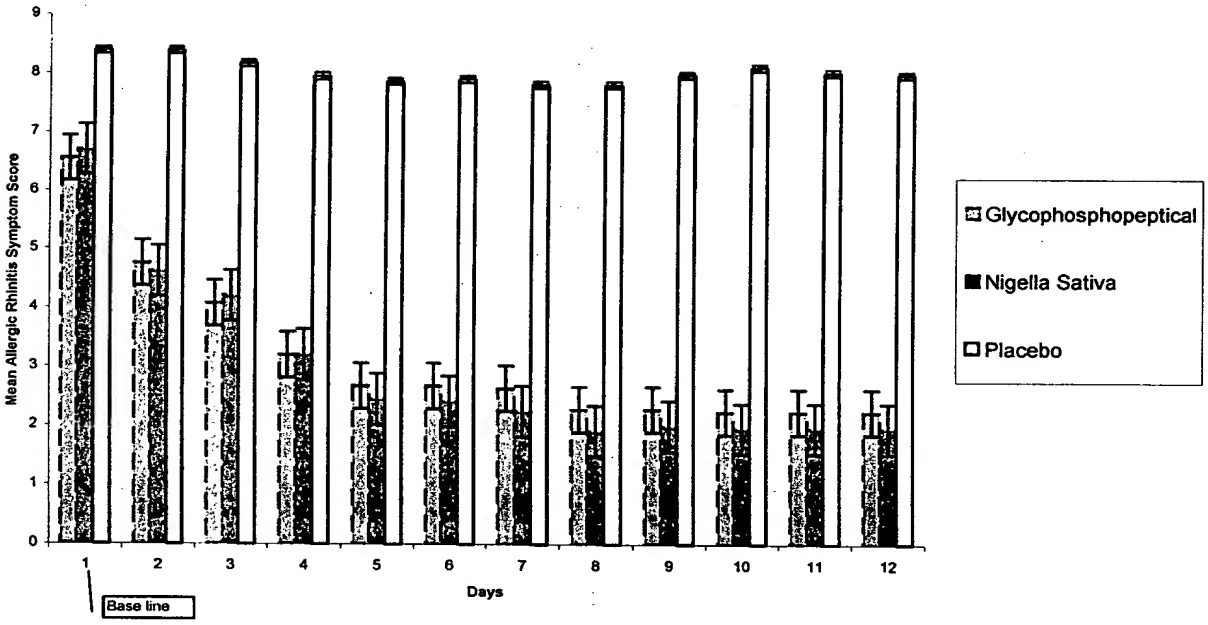

Figure 1. Mean of allergic rhinitis symptom score for pure seeds of *Nigella sativa* (n=24), glycophosphopeptical (n=25), and placebo (n=23). The scores are based on the symptoms of running nose, frequency of sneezing, and nasal obstruction. The values are reported as mean +/- SE.

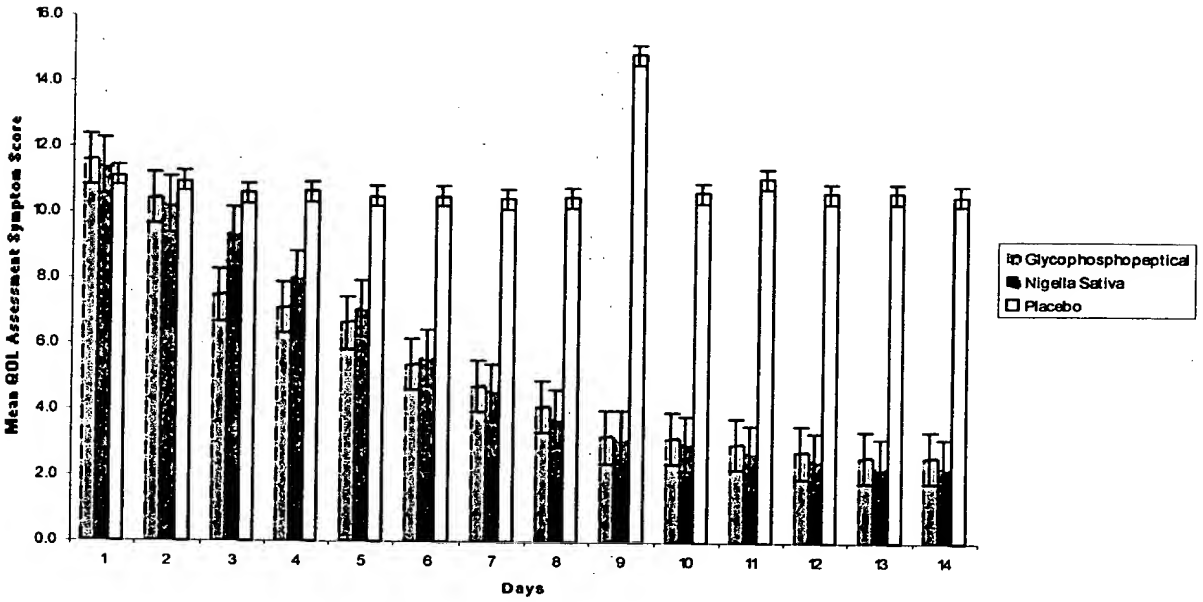

Figure 2. Mean of allergic rhinitis to quality of life assessment (QOL) score for patients treated with pure seeds of *Nigella sativa* (n=24), glycophosphopeptical (n=25), and placebo (n=23). The quality of life assessment (QOL) scores are based on symptoms, which interfere with the daily activities, and other symptoms such as: disturbance of sleep, loss of sense of smell, and psychological suffering. The values are reported as mean +/- SE.

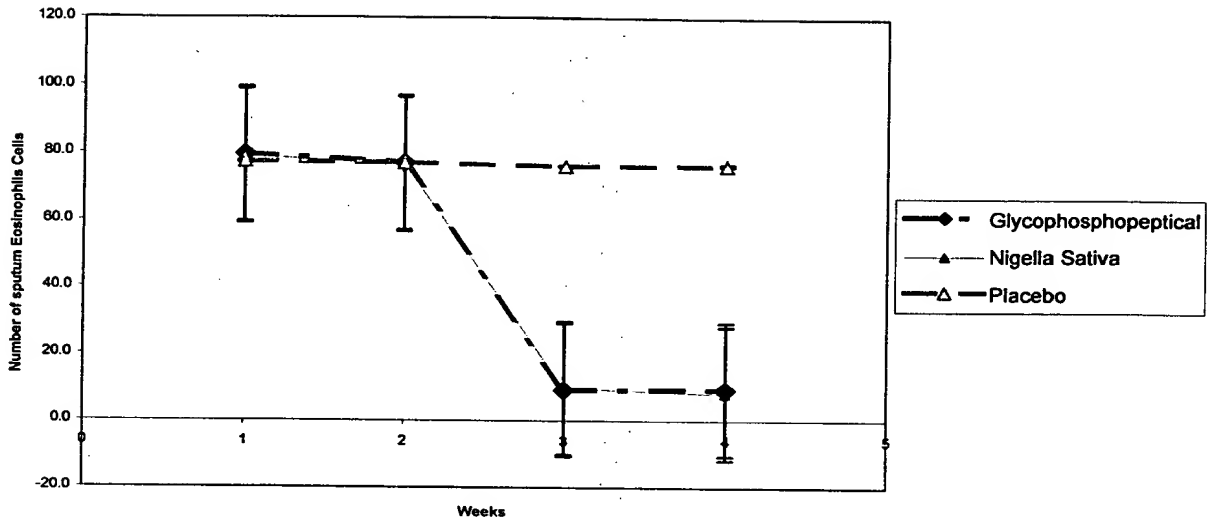

Figure 3. The effect of treatments with *Nigella sativa* (n=23), glycophosphopeptical (n=21), and placebo (n=21) on sputum eosinophils in patients with severe chronic asthma. The values are reported as mean +/- SE.

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Therapeutic points of intervention and clinical implications: role of desloratadine

Desloratadine, a potent, once-daily, orally active, nonsedating, histamine H_1-receptor antagonist, inhibits the release of histamine and other inflammatory mediators. Once-daily desloratadine therapy rapidly reduces the symptoms of perennial allergic rhinitis and seasonal allergic rhinitis (SAR), reduces the use of inhaled albuterol by patients with SAR and concomitant asthma, and improves symptoms and quality of life in patients with chronic idiopathic urticaria. An open-label, observational study in SAR patients revealed that desloratadine therapy significantly reduced nasal, ocular, dermal, asthma, and total symptoms, and enabled half of the patients with concomitant asthma to reduce their use of asthma medications. Globally, more than 91% of patients and physicians judged desloratadine to have excellent or good efficacy, and more than 98% judged it to have excellent or good tolerability. Furthermore, desloratadine therapy improved quality of life, decreasing by more than 10-fold the percentage of patients whose daily activities and/or sleep were moderately or severely affected by SAR. Allergic rhinitis, a major chronic airway disease that is a risk factor for asthma, warrants extended diagnostic procedures and well-tolerated therapy that encompasses the entire airway, addresses multiple steps in the allergic inflammatory cascade, and is effective on nasal, ocular, dermal, asthma, and total symptoms.

C. Bachert

ENT Department, University Hospital Ghent, Ghent, Belgium

Key words: allergic rhinitis, asthma, desloratadine

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The release of histamine and other proinflammatory mediators from mast cells is central to the early phase of an IgE-dependent allergic reaction and contributes to late and ongoing allergic inflammation (1). Histamine H_1-receptor antagonists (antihistamines) block histamine H_1-receptor activity. Most classical and newer antihistamines have additional antiallergic properties that differ depending on the molecule (2). The variability between antihistamines in H_1-receptor antagonist activity and antiallergic effects, possibly in combination with differences in lipophilicity and tissue deposition, result in different degrees of efficacy on skin, nose, eye, and lung symptoms (2).

Allergic rhinitis is the most prevalent manifestation of IgE-dependent allergy. It affects about 10% to 20% of the general population in many developed countries (3), and the prevalence has increased in some geographic areas in the last decades (4). Allergic rhinoconjunctivitis is common in children worldwide, with the 1-year prevalence across centres varying from 0.8% to 14.9% among children aged 6–7 years and from 1.4% to 39.7% among 13–14-year-old adolescents (5). As expressed in the European Academy of Allergology and Clinical Immunology (EAACI) consensus statement, allergic rhinitis is underestimated as a cause of suffering and impaired quality of life and may contribute to learning problems and sleep disturbances (3).

Desloratadine is a potent, once-daily, orally active, nonsedating H_1-receptor antagonist. This article reviews desloratadine preclinical and clinical allergy data. It details the multiple points of intervention of desloratadine in the allergy cascade and describes the utility of the medication in the treatment of allergic disease, focusing on seasonal allergic rhinitis (SAR), including the quality of life of patients with SAR.

Preclinical data

Desloratadine inhibits the release or generation of histamine and many other inflammatory mediators, resulting in a positive impact on early-phase reaction symptoms and chronic inflammation (Table 1).

Antihistaminic profile

The antihistaminic potency of desloratadine has been demonstrated by using the human H_1-receptor cloned and expressed in Chinese hamster ovary cells (Table 2). According to these data, desloratadine is one of the most potent antihistamines currently available, with a potency that is 80–115 times higher

Table 1 The allergic inflammatory cascade: desloratadine intervention points, actions, and theoretical consequences (*in vitro* data)

Cell population	Target (action)	Theoretical consequence
Mast cell and/or basophil	Histamine (↓ release) Leukotriene C_4 (↓ release) Prostaglandin D_2 (↓ release) Tryptase (↓ release)	↓ Early-phase response severity
Mast cell and/or basophil	TNF-α-induced RANTES (↓ release) GM-CSF (↓ release) IL-3 (↓ release) IL-4 (↓ release/generation) IL-13 (↓ release) IL-6 (↓ release) IL-8 (↓ release)	↓ IFC chemotaxis, recruitment, activation, and adhesion
Epithelial cell	Histamine-induced ICAM-1 (↓ expression) TNF-α-induced RANTES (↓ release)	↓ IFC adhesion ↓ Eosinophil infiltration
Endothelial cell	IL-6 (↓ release) IL-8 (↓ release) Histamine-induced P-selectin (↓ expression)	↓ Leukocyte adhesion to endothelial cells and diapedesis
Monocyte (PBMC)	IL-1β (↓ release) IL-18 (↓ release) IL-5 (↓ release)	↓ IFC migration and activation
Th2 T lymphocyte (from allergic patients)	IL-4 (↓ release) IL-13 (↓ release) IL-5 (↓ release)	↓ IFC recruitment and activation
Lymphocyte (T cell, B cell and/or NK cell)	Co-stimulatory molecule Ox40 (CD134) (↓ expression)	↓ Allergen-stimulated lymphocyte proliferation
Eosinophil (from allergic patients)	PAF (↓ activity) TNF-α (↓ activity) Superoxide (↓ synthesis)	↓ Chemotaxis ↓ Adhesion ↓ Cytotoxicity
Neutrophil	CD11b (↓ expression)	↓ Chemotaxis/cytotoxicity

ICAM-1, intracellular adhesion molecule-1; IFC, inflammatory cell; IL, interleukin; PAF, platelet-activating factor; RANTES (regulated on activation, normal T cell expressed and secreted); TNF, tumour necrosis factor (6–8,10–21).

Table 2 Comparative potency to block responses of chinese hamster ovary (CHO) cells containing a cloned human H_1-receptor (6)

Antihistamine	Potency (K_b, nM)	Relative rank (1–9)
Desloratadine	0.2	1
Loratadine	16	7
Azelastine	0.3	2
Mizolastine	0.8	3–4
Astemizole	0.8	3–4
Terfenadine	3	5
Ebastine	7.9	6
Cetirizine	19	8
Fexofenadine	23	9

K_b, base ionization constant.

than loratadine, cetirizine, or fexofenadine *in vitro* (6).

Several animal models have further demonstrated the antihistaminic potency of desloratadine (7). Desloratadine was approximately four times more potent than loratadine in inhibiting histamine-induced paw oedema in the mouse (ED_{50} of 0.15 mg/kg and 0.60 mg/kg, respectively; $P < 0.05$). Guinea pigs were protected from the lethal effects of histamine with an ED_{50} of desloratadine (0.15 mg/kg) two-fold lower than that of loratadine (0.37 mg/kg). The increase in microvascular permeability in response to a histamine challenge to the upper airway of guinea pigs was inhibited with topical desloratadine in a dose one-tenth that of loratadine ($ED_{50} = 0.9$ μg *vs.* 8.7 μg, respectively).

Anti-inflammatory profile

In vitro studies have shown that, in addition to its antihistaminic properties, desloratadine has direct effects on inflammatory mediators in human cells. Desloratadine inhibited IgE-mediated release of histamine, tryptase, leukotriene C_4 (LTC_4), and prostaglandin D_2 (PGD_2) from mast cells, and histamine and LTC_4 release were inhibited from peripheral blood basophils (8). Desloratadine also inhibited the release of interleukin (IL)-3, tumour necrosis factor-α (TNF-α), and granulocyte macrophage colony-stimulating factor (GM-CSF) from human mast cells (9). In cells with high-affinity receptors for IgE (*Fc*εRI) desloratadine reduced release of the TNF-α-induced chemokine, RANTES (regulated on activation, normal T cell expressed and secreted) (10).

Preincubation of mast cells and basophils with desloratadine suppressed IL-6 release by up to 40% and IL-8 release by up to 50%, the magnitude of suppression being concentration-dependent (11). Desloratadine inhibited both IgE-mediated and non-IgE-mediated release of the cytokines IL-4 and IL-13 by human basophils. It is important to

note that desloratadine was six to seven times more potent an inhibitor of IL-4 and IL-13 than of histamine or LTC_4 (12). Pretreatment of human basophils with desloratadine inhibited IL-4 expression by up to 80% (12).

Desloratadine also targets the proinflammatory mediators from other human cells involved in IgE allergic reactions. Incubation with desloratadine at a concentration of 10 μM significantly inhibited the induction of intracellular adhesion molecule-1 (ICAM-1), an index of airway cell activation, in human nasal epithelial cells (13). Desloratadine inhibited the TNF-α-stimulated release of RANTES in epithelial cells, suggesting the potential to decrease the infiltration of airways by eosinophils (10,14). Desloratadine inhibited the endothelial cell release of IL-6 and IL-8 (11), and of P-selectin (15), the latter of which is involved in both the adhesion of leukocytes to the endothelial cell and in diapedesis.

The lipopolysaccharide-induced release of IL-1β and IL-18 from human peripheral blood mononuclear cells (PBMCs) was inhibited by approximately one-third by preincubation with desloratadine at concentrations of 10^{-7}–10^{-5} M for 2 h (14). Culture of PBMCs from patients with allergy to wasp venom in the presence of desloratadine (1–10 μg/ml) significantly decreased the secretion of IL-5 (16).

In allergen-activated/human lymphocytes (T cells, B cells, and/or natural killer cells), desloratadine significantly reduced expression of the costimulatory molecule Ox40 (CD134) and decreased lymphocyte proliferation (17). *In vitro* studies show that the release of cytokines, chemokines, and adhesion molecules is diminished by desloratadine (18). Culture of leukocytes from allergic patients for 2 weeks with desloratadine (10^{-7} M) led to a reduction in the stimulated secretion from Th2 T lymphocytes of IL-4 (by 55% (allergic rhinitis) and 39% (allergic asthma), respectively), of IL-13 (by approximately 40% in each group), and of IL-5 (by 61% in each group) (19). In contrast, culture of leukocytes from healthy controls with no history of allergy in the presence of desloratadine decreased the stimulated secretion of IL-4 by only 19% and increased that of IL-13 and IL-5 (19). In a study of eosinophils from patients with allergic rhinitis or allergic asthma, desloratadine (10^{-7}–10^{-5} M/l) inhibited eosinophil chemotaxis induced by platelet-activating factor, eosinophil adhesion induced by TNF-α, and superoxide generation induced spontaneously and by phorbol myristate acetate (20). Preincubation of neutrophils with desloratadine (10^{3}–10^{0} mM) for 1 h resulted in a down-regulation of the constitutive and inductive expression of CD11b and a decrease in the chemotactic and cytotoxic response to chemoattractants, such as aqueous pollen extracts (21).

Anti-inflammatory effects in atopic patients

In preliminary results from placebo-controlled studies, desloratadine 20 mg once daily appeared to modulate allergic inflammation *in vivo* in subjects with SAR. Supernatants of PBMCs from subjects given desloratadine for 1 week showed increased expression of interferon-γ (INF-γ) (Th1 cytokine) and decreased expression of IL-4, IL-5, and IL-10 (Th2 cytokines), compared with those of subjects administered placebo (22). During allergy season, a decrease in circulating levels of eosinophil and basophil progenitors is seen as these leave the bloodstream and enter allergically activated tissues. At the peak of ragweed season, after 2 weeks of dosing, placebo recipients showed the expected fall in the number of circulating eosinophil and basophil progenitors, which was greater than the fall in desloratadine recipients ($P = 0.013$) (23). These cells, harvested and tested *in vitro*, also released less histamine per colony when the patient was pretreated with desloratadine. The results of these studies suggest that desloratadine both modifies the cytokine balance and alters cellular shuttling in people with SAR.

Clinical efficacy

The clinical efficacy of desloratadine therapy is documented by the study of patients with allergic rhinitis and of patients with chronic idiopathic urticaria. In controlled clinical studies, once-daily desloratadine therapy relieved the nasal and non-nasal symptoms of perennial allergic rhinitis (24) and SAR (25), including congestion (7). In patients with SAR and concomitant asthma, once-daily desloratadine therapy reduced the use of inhaled albuterol (7). Furthermore, once-daily desloratadine therapy in patients with chronic idiopathic urticaria reduced pruritus, number of hives, size of the largest hive, and interference with sleep and daily activities (26). The use of desloratadine therapy in the treatment of SAR, including published clinical data and recent postmarketing data, is reviewed below.

Controlled clinical studies of desloratadine therapy in SAR

Two multicentre, randomized, double-blind studies, one conducted in the spring allergy season ($n = 346$) and one conducted in the autumn allergy season ($n = 328$) in the USA, compared the efficacy of desloratadine 5 mg and placebo, once daily for 14 days, in patients aged 12 years or older with moderate-to-severe SAR (25). The total symptom score (TSS) was assessed in the morning and the evening, and reflected the patient's recollection of

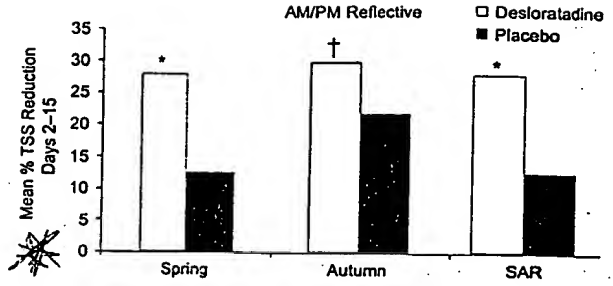

Figure 1 Desloratadine therapy in seasonal allergic rhinitis (SAR): mean percentage reduction in total reflective symptom scores (TSS) from baseline (* $P < 0.01$; † $P = 0.02$). Adapted from (25,30).

symptoms during the previous 12 h. The mean change from baseline in the reflective TSS averaged over the 2-week study period was the primary efficacy assessment. Desloratadine therapy produced a 28% (spring) and 30% (autumn) reduction in the TSS (Fig. 1) compared with 13% ($P < 0.01$ *vs.* desloratadine) and 22% ($P = 0.02$ *vs.* desloratadine) reduction with placebo. Most adverse events were mild to moderate in severity; headache, the most frequently reported adverse event, and somnolence, commonly associated with older antihistamines, occurred at similar rates with placebo and desloratadine treatment.

Relief of nasal congestion/stuffiness by once-daily desloratadine therapy was demonstrated in three multicentre, double-blind, placebo-controlled studies of patients with symptomatic SAR ($n = 300$ in each) (7). Twice daily, in the morning and the evening, nasal congestion/stuffiness was scored over a range of 0 (none) to 3 (severe), with the score refecting the patient's recollection of symptoms over the previous 12 h. Desloratadine therapy reduced mean baseline scores by 21.3–23.5%, which was significant compared with the 13.6–16.2% reduction in the placebo groups ($P < 0.05$).

Desloratadine therapy was compared with placebo in two multicentre, double-blind studies of patients with SAR and mild-to-moderate asthma, the results of which were pooled (27). Participants had at least a 2-year history of SAR and asthma, symptoms for at least 3 days before baseline assessment, and a forced expiratory volume in 1 s (FEV_1) of at least 70% of predicted value. Compared with placebo, desloratadine therapy was associated with a significant decrease from baseline in the mean asthma TSS beginning with the first dose and maintained over the 4 weeks of the study. The use of inhaled β_2-agonists was reduced significantly more with desloratadine therapy than with placebo.

Desloratadine, a nonsedating antihistamine, improves SAR symptoms without adversely affecting cognitive function. In a controlled comparison, patients with SAR who were symptomatic after priming with ragweed pollen were randomized to a single dose of desloratadine ($n = 81$), diphenhydramine ($n = 84$), or placebo ($n = 83$) (28). A battery of repeatable, automated, neuropsychological tests was administered at baseline and 90 min after dosing. There was no difference between desloratadine and placebo in any cognitive parameter, but the cognitive function of patients treated with diphenhydramine was significantly worsened, to a degree comparable to that caused by conditions such as mild head injury or migraine. Furthermore, on all parameters of vigilance, diphenhydramine produced clinically meaningful decrements, while desloratadine produced no significant change.

Postmarketing study of desloratadine therapy in SAR

At the launch of desloratadine in Germany in January 2001, an open-label, observational study was initiated to collect data on almost 50 000 patients with SAR (29). Data were recorded using a simple, modified, case report form. At the beginning and end of desloratadine therapy, patients used 4-point scales to separately rate the severity of nasal symptoms (nasal congestion, runny nose, sneezing/itching), ocular symptoms (watery eyes, burning/itching, redness), dermal symptoms (itching, hives/pustules, dryness), and asthma symptoms (wheeze, breathlessness, chest tightness, cough). Sum scores were calculated for each symptom category (nasal, ocular, dermal, asthma), and a TSS was calculated for the sum of individual category scores.

A total of 47 953 people participated in the study, 42% men and 58% women (mean age 38.8 years). Dermal symptoms were present in 39.3%. Use of asthma medication was reported by 7021 (15%) patients, but 19 512 (41.5%) reported at least one asthma symptom at baseline. The mean duration of desloratadine therapy was 38.4 days, and compliance was rated as good to excellent in 98% of patients.

Desloratadine 5 mg once daily significantly reduced sum scores for nasal symptoms, ocular symptoms, dermal symptoms, and asthma symptoms, and significantly reduced the TSS ($P = 0.0001$) (Table 3). The percentage of patients with moderate or severe nasal symptoms decreased more than tenfold, from 71.6% to 5.2% for rhinorrhoea, 73.7% to 5.2% for sneezing/itching, and 67.2% to 6.2% for nasal congestion following desloratadine. At the end of treatment, more than half (55.1%) of the patients had no nasal congestion compared with only 13.6% before treatment. Desloratadine therapy enabled half of the SAR patients with asthma symptoms at baseline to reduce their use of asthma medication.

Table 3 Decrease in SAR symptom scores with desloratadine therapy

Symptoms	Symptom score		
	Pretreatment	Post-treatment	Decrease (%)
Nasal	5.45	1.41	74.13*
Ocular	4.34	0.84	80.65*
Dermal	1.24	0.34	72.58*
Asthma†	3.68	1.01	72.55*
Total symptom score (TSS)	12.43	2.99	75.95*

*$P = 0.0001$.
†Subset of patients using asthma medication ($n = 7021$).
Reproduced from (29).

Patients and physicians rated the global efficacy, tolerability, and onset of action of desloratadine therapy compared with previously administered antihistamines including loratadine, cetirizine, fexofenadine, and others. Previous antihistamine therapy had been received by 17 513 (36.5%) patients, which was rated excellent or good for efficacy by 40.7% of patients. In contrast, more than 91% of patients (91.2%) and physicians (92.6%) judged desloratadine therapy excellent or good for efficacy. More than 98% of patients (98.5%) and physicians (98.9%) judged desloratadine excellent or good for tolerability, reflecting the low adverse event rate (0.44%). Onset of action of desloratadine was rated as faster than previous antihistamine therapy by 64.1% of doctors and 65.7% of patients.

Desloratadine improved quality of life by decreasing the impact of SAR symptoms on daily activities and sleep. During desloratadine therapy, SAR caused a moderate or severe interference with daily activities in only 3.0% of patients compared with 40.0% of patients before therapy and a moderate or severe disruption of sleep in only 2.6% of patients compared with 34.3% before therapy (Fig. 2).

Points of intervention and implications

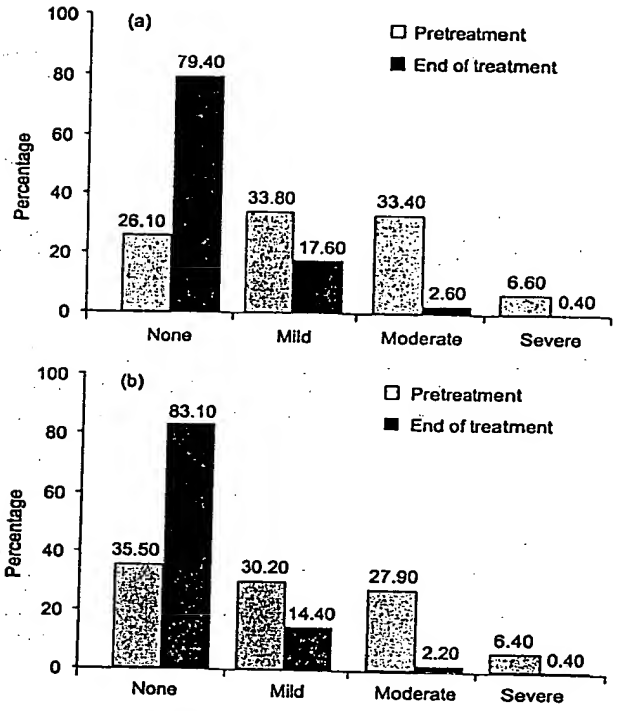

Figure 2 Desloratadine therapy in seasonal allergic rhinitis (SAR): SAR-related interference with (a) daily activities and (b) sleep disturbance before treatment and at the end of treatment. Adapted from (31).

Conclusions

Allergic rhinitis is a major chronic airway disease that negatively affects quality of life and represents a risk factor for asthma. Desloratadine, a potent, once-daily, orally active, nonsedating, histamine H_1-receptor antagonist inhibits multiple steps in the allergic inflammatory cascade *in vitro*. It is effective on nasal, ocular, dermal, asthma, and total symptoms, and is well tolerated. The inhibition of multiple steps in the allergy inflammatory cascade may provide an explanation for the broad clinical activity of desloratadine in the clinical setting. In SAR, desloratadine improves nasal, ocular, dermal, and asthma symptoms, decreases the use of asthma medication, and improves quality of life.

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SUPPLEMENT

Desloratadine: A new approach in the treatment of allergy as a systemic disease – pharmacology and clinical overview

GUEST EDITOR:
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Decongestant efficacy of desloratadine in patients with seasonal allergic rhinitis

Recent advances in experimental immunologic approaches to seasonal allergic rhinitis (SAR) have led to a shift in the concepts of its pathogenesis. The conventional view of SAR as a local response to inhaled allergens has largely given way to a new view of this disorder as a systemic condition with local tissue manifestations. This concept, together with an increasing recognition of specific mediators' distinct roles in driving the early- and late-phase allergic responses, has opened multiple lines of therapeutic attack within the allergic cascade. Potent inhibition of inflammatory mediator release at distinct points in this cascade is conferred by desloratadine. In addition to the familiar range of SAR symptoms amenable to antihistamine therapy, desloratadine uniquely attenuates patient ratings of nasal congestion. This novel, nonsedating histamine H_1-receptor antagonist is the only once-daily antiallergic product with a consistent decongestant effect that begins within hours of the first morning dose and is sustained for the entire treatment period.

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Key words: allergic cascade; allergic rhinitis; congestion; desloratadine.

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Progress in the immunohistology of seasonal allergic rhinitis (SAR), together with the development of refined nasal-challenge models, has substantially clarified the pathogenesis of this disorder, particularly within the past 15 years (1–4). Elucidation of the complex effector mechanisms underlying SAR and other inflammatory conditions has occasioned a fundamental departure in both experimental and clinical approaches to SAR. Once viewed as a predominantly local reaction to inhaled allergens, SAR is increasingly being seen as a systemic condition with diverse, often comorbid, local effects on the airways.

This emerging approach to SAR as a systemic condition has also introduced the concept of mediator specificity: the distinct roles of cytokines, chemokines, adhesion molecules, and other mediators in regulating complex interactions among effector cells. These include mast cells, basophils, eosinophils, T cells, and other leukocytes, as well as epithelial and endothelial cells. The proinflammatory mediators include histamine; lipid mediators, such as leukotrienes (e.g., LTC_4) and prostaglandins (e.g., PGE_2); cytokines, such as interleukins and tumor necrosis factor-alpha (TNF-α); chemokines, such as eotaxins and RANTES (regulated on activation, normal T-cell expressed and secreted); and adhesion molecules, such as the selectins and intercellular adhesion molecule-1 (ICAM-1).

This conceptual shift enables targeting of multiple points of therapeutic attack within the allergic cascade. For instance, interleukin-1β (IL-1β) and TNF-α have been isolated in nasal secretions from patients with allergic rhinitis (AR) (5). Each of these cytokines upregulates allergen-induced expression of the adhesion molecule E-selectin by endothelial cells, enabling leukocytes to interact with these cells and migrate across the airway vascular endothelium. Work involving nasal mucosal cells from patients with AR has demonstrated that exposure of these cells to soluble IL-1 receptors and TNF-binding proteins (5) markedly suppresses E-selectin induction *in vitro*. These approaches, along with others, such as anti-IL-5 monoclonal antibodies, sIL-4 receptors, and anti-VLA-4 (very late activation antigen-4), thus represent plausible lines of potential pharmacologic attack.

Interestingly, recent experimental and clinical work indicates that desloratadine potently inhibits the allergic cascade at many points and significantly relieves symptoms of SAR, including nasal congestion.

Overview of the pathophysiology of nasal congestion

Key effector mechanisms in the allergic cascade

The complex immunopathogenesis of type I allergic inflammation in the nose is illustrated in Fig. 1 (courtesy of Dr Ruby Pawankar) (6). Acute symptoms typically experienced by SAR sufferers within the first 30 min (e.g., wheeze, cough, rhinorrhea, and congestion) result when the host initiates the acute-phase allergic response after inhalation of pollen allergens (e.g., ragweed) (7). However, after diffusing across the nasal mucosa, these allergens – through the actions of

Table 1. Affinity constants obtained from ^{3}H-labeled pyrilamine binding to human recombinant histamine H_1 receptor from membranes of Chinese hamster ovary cells

Compound	K_i (nM ± SEM	Relative potency
Desloratadine	0.87 ± 0.1	201
Cetirizine	47.2 ± 10	3.7
Ebastine	51.7 ± 6.8	3.4
Fexofenadine	175 ± 68	1.0
Loratadine	138 ± 23	1.2
Mizolastine	22 ± 6	8.0
Pyrilamine	1.7 ± 0.1	103
Terfenadine	40 ± 4.6	4.4

SEM: standard error of mean.
With permission of Anthes et al. (10).

buffer) inhibition of both IgE-dependent and independent release of histamine from mixed peripheral-leukocyte preparations (11). Similar trends were observed when either anti-IgE-activated human basophils or 2,4-dinitrophenyl (DNP)-triggered rat basophilic leukemia (RBL-2H3) cells were incubated with desloratadine or loratadine at concentrations exceeding 2 and 7 μM, respectively (12).

The mechanism underlying these effects might involve desloratadine's capacity to mobilize cytosolic Ca^{2+} stores and attenuate the Ca^{2+} influx necessary for IgE-mediated degranulation and, with it, release of histamine and other proinflammatory mediators from effector cells (e.g., mast cells and basophils) (13). In a CHO line, desloratadine was a more potent antagonist of Ca^{2+} flux than cetirizine, fexofenadine, terfenadine, astemizole, or loratadine (14).

Second, Genovese et al. (15) observed that preincubation of cell cultures with desloratadine at pharmacologic concentrations of approximately 10 μM significantly inhibited the anti-FcεR1-induced release of histamine and LTC_4, as well as eicosanoid PGD_2 and tryptase, from basophils and mast cells derived from human skin and lung tissues.

Third, *in vitro* studies also showed that desloratadine markedly diminished the release of numerous cytokines, chemokines, and adhesion molecules that promote the proliferation and differentiation, as well as the tissue infiltration and recruitment, of key effector cells. For instance, incubation of epithelial cells (from nasal turbinates or polyps) with desloratadine at a concentration of 10 μM reduced histamine-induced membrane expression of ICAM-1 and human leukocyte class II (HLA-DR) antigen, two indices of airway epithelial-cell activation (16).

Desloratadine's potent anti-inflammatory effects in human mast cells, as well as basophils and endothelial cells, were demonstrated by the agent's inhibitory effects on histamine- or phorbol myristate acetate (PMA)-stimulated IL-6 and IL-8 release (17), which reached 50% at a concentration of approximately two to five orders of magnitude lower than that of

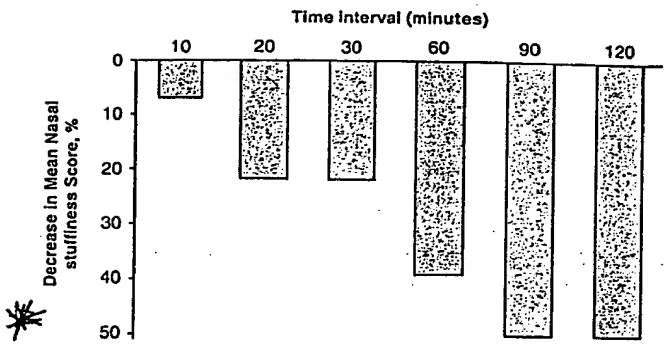

Figure 3. Percent change in nasal stuffiness scores (maximum: 3) after allergen challenge and desloratadine dosing on day 1 among 2-point responder group ($n = 14$). With permission of Horak et al. (25).

loratadine in a human umbilical-vein endothelial cell (HUVEC) preparation (18). At nanomolar concentrations, desloratadine also significantly inhibited expression by HUVECs of P-selectin (18), which promotes leukocyte adhesion to endothelial cells and diapedesis.

Inhibition by desloratadine of TNF-α-stimulated release of RANTES by epithelial cells *in vitro* (19) suggests that desloratadine can potentially diminish airways infiltration by eosinophils. Three inflammatory functions were attenuated by desloratadine when introduced to eosinophils isolated from 10 patients with AR or AR plus asthma (20).

First, chemotaxis of human eosinophils in response to the lipid mediator platelet-activating factor was significantly suppressed by des- -adine at pharmacologic concentrations, reaching a maximum of 36% (±8%) at 10 μM (Fig. 2).

Second, at the same concentration, desloratadine also induced maximal inhibition (27% ± 5%) of TNF-α-stimulated adhesion of ^{51} Cr-labeled eosinophils to HUVECs, an effect that was also significantly dose related (Fig. 2).

Third, incubation of eosinophils with desloratadine (10 μM) elicited significant declines (as compared with

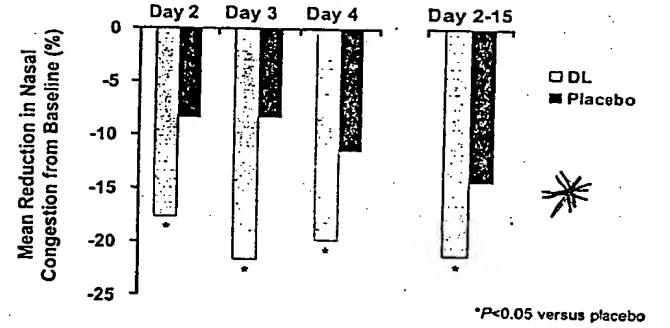

Figure 4. Percent change from baseline in nasal congestion scores for desloratadine 5 mg and placebo. With permission of Prenner et al. (21).

Potential clinical implications

The high prevalence of nasal congestion, together with its adverse effect on quality of life, place desloratadine's consistent, sustained decongestant effects in appropriate clinical perspective. At least one 14-day bout with nasal congestion in the prior year was reported in about 17% of questionnaires in a British household survey (30). Furthermore, according to one estimate (31), 47–64% of SAR or PAR subjects suffer from nasal obstruction.

Patients with nasal congestion due to AR are nearly twice as likely to report sleep-disordered respiration (32), and such nighttime symptoms render allergy sufferers significantly more likely to report daytime sleepiness. This problem is of untold dimensions because many patients ascribe their daytime somnolence to medication side-effects. In clinical trials, desloratadine consistently diminished mean nasal congestion symptom severity scores from about 2.3, which was within the range associated with sleep disorders.

The potential physiologic benefits of desloratadine's decongestant effects are at least twofold. First, mucosal swelling and inflammation could conceivably limit the access of other medications to absorptive mucosal surface area and, if severe, could even limit the bioavailability of these agents (33). Second, and perhaps more important, patients suffering from nasal congestion may be more prone to mouth breathing, which can, in turn, promote inhalation of airborne allergens – with introduction of these allergens to the lower airway. These events may contribute to the pathogenesis of AR in certain susceptible individuals (34).

Conclusions

In summary, increasing recognition of the complex interactions in the allergic cascade has prompted a new concept in which the mechanisms of allergy are viewed as aspects of a systemic condition with local, frequently comorbid, tissue effects. In the clinical management of SAR, this approach opens a number of potential lines of therapeutic attack on mediators in the allergic cascade. The novel, nonsedating histamine H_1-receptor antagonist, desloratadine, potentially inhibits the allergic cascade at various points, including both early- and late-phase responses. Furthermore, only desloratadine has shown consistent, significant 24-h decongestant effects with an onset in minutes to hours of the first dose and persisting with daily dosing for up to 4 weeks.

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SUPPLEMENT

Expanding the Anti-Allergy Therapeutic Horizon

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Advances in allergy management

Our understanding of the pathophysiology of allergy has moved to the molecular level, while study of epidemiology and genetics has revealed risks of developing allergies based on environmental and genetic profiles, and pharmacoeconomic data have enabled accurate measurement of the immense burden of allergic disease. These advances in allergy research have affected its management, particularly the search for new antiallergy therapies. New therapies should intervene in the systemic allergy inflammatory cascade and provide clinical efficacy that extends to multiple allergic disease states. In addition, these new therapies should present no additional safety issues, offer improvements over existing therapies, and have an impact on disease-impaired quality of life. *In vitro* studies show that desloratadine, a new, once-daily, nonsedating, selective histamine H_1-receptor antagonist, blocks the systemic allergy cascade at multiple points. Desloratadine 5 mg once daily relieves the symptoms of chronic idiopathic urticaria and of both seasonal (SAR) and perennial allergic rhinitis. In patients with concomitant asthma and SAR, asthma symptoms are relieved and β_2-agonist medication use is decreased by desloratadine. Unlike many other second-generation histamine H_1-receptor antagonists, desloratadine provides the added benefit of efficacy against nasal obstruction in SAR. Desloratadine improves quality of life by decreasing the impact of allergic symptoms on sleep and on daily activities.

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Key words: allergic rhinitis, asthma, desloratadine, pathophysiology, pharmacoeconomics

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Since the early 1990s, several advances have been made in the biomedical research and medical treatment of allergy. Understanding of the pathophysiology of allergy has moved to the molecular level, enabling the discovery of new treatment targets and new tools to assess drug efficacy and safety. The fields of epidemiology and genetics have converged to reveal risks of developing disease, based on environmental and genetic profiles. This may allow the identification of those at risk before the disease is clinically apparent. Pharmacoeconomic studies allow both the costs of illness and the benefits of wellness to be measured, thus enabling accurate measurement of the burden of allergic disease.

In this article, the effect of advances in the biomedical research of allergy is illustrated with data on desloratadine, a new selective histamine H_1-receptor antagonist.

Advances in biomedical research of allergy

Pathophysiology

Allergy is now understood to be a systemic inflammatory disease (Fig. 1). Multiple inflammatory mediators have been identified (1–3). These include cytokines, such as the interleukins (IL-3, IL-4, IL-5, IL-9, and IL-13), which promote the production of immunoglobulin E (IgE), airway hyperresponsiveness, the overproduction of mucus, and the development of eosinophils and mast cells, all of which are associated with chronic allergic inflammation. Other inflammatory mediators that have been identified are chemokines, such as 1) eotaxins, 2) RANTES (regulated on activation, normal T cell expressed and secreted), 3) monocyte chemoattractant proteins (e.g. MCP-2 and MCP-3) (3), and 4) thymus- and activation-regulated chemokine (TARC) (4,5). These chemotactic cytokines have the ability to attract and activate leukocytes. Adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), vascular cellular adhesion molecule-1 (VCAM-1), and selectins (e.g. selectin-P, selectin-E) (3,6), are surface ligands that mediate cell-to-cell adhesion and diapedesis. Chemokines recruit eosinophils and basophils, and adhesion molecules promote the accumulation of inflammatory cells, processes that are central to the maintenance of chronic allergic inflammation.

Epidemiology and genetics

The prevalence of allergic rhinitis varies widely, from 1% to 40%, according to the population studied and to the conditions and methods of assessment (3). Prevalence varies widely in different

several interleukins (IL-1β, IL-3, IL-4, IL-5, IL-6, IL-8, IL-13, IL-18), and platelet-activating factor are among the mediators affected by desloratadine *in vitro*. Furthermore, *in vitro* studies revealed that desloratadine decreases the chemotaxis and activation of eosinophils and neutrophils and significantly down-regulates the expression of CD11b on polymorphonuclear leukocytes, suggesting inhibition of transmigratory capacity (58). In atopic patients with SAR who were administered desloratadine 20 mg daily ($n = 13$) for a week, supernatants of peripheral blood mononuclear cells (PBMCs) showed increased expression of interferon-γ (type 1 cytokine) and decreased expression of IL-4, IL-5, and IL-10 (type 2 cytokines), compared with supernatants of PBMCs of those administered placebo ($n = 6$) (59). Also, desloratadine recipients had increased natural killer (NK) cell activity and decreased circulating basophils, eosinophils, and serum VCAM-1.

Efficacy against multiple disease manifestations of allergy

Given the systemic nature of allergy, targeting of the allergy cascade should result in efficacy against multiple disease manifestations. Desloratadine 5 mg once daily is effective in the treatment of both seasonal allergic rhinitis (SAR) and perennial allergic rhinitis. In people with SAR and symptoms of asthma, desloratadine relieves asthma symptoms and decreases the use of rescue medication. Desloratadine also relieves the symptoms of chronic idiopathic urticaria (CIU), 5 mg once daily being superior to placebo in controlling pruritus and total symptoms beginning with the first dose and throughout the 6 weeks of the study (Fig. 2) (60).

Desloratadine 5 mg once daily relieves the nasal and non-nasal symptoms of allergic rhinitis,

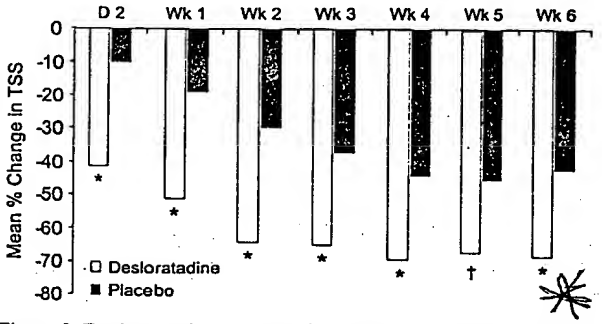

Figure 2 Desloratadine therapy in chronic idiopathic urticaria (CIU), as demonstrated in a double-blind, randomized, placebo-controlled, multicentre trial that included 190 patients aged 12 years and above with at least a 6-week history of CIU and experiencing a flare of at least moderate severity. Mean percentage reduction in total symptom score (TSS) from baseline. *$P < 0.001$; †$P = 0.002$. Reproduced with permission from (69).

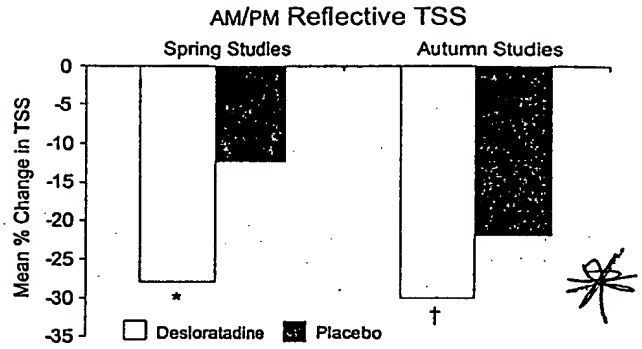

Figure 3 Desloratadine therapy in seasonal allergic rhinitis. Mean percentage reduction in morning/evening reflective total symptom score (TSS) from baseline. *$P < 0.01$; †$P = 0.02$. Adapted from (61).

both SAR and perennial allergic rhinitis. Two multicentre, randomized, double-blind, placebo-controlled, parallel-group investigations were conducted in patients with SAR, one during the spring allergy season (172 and 174 patients in the desloratadine and placebo groups, respectively) and one during the autumn allergy season (164 patients in each group) (61). Patients aged 12 years or older with a minimum 2-year history of SAR were randomized to desloratadine 5 mg or placebo once daily for 14 days, following a 3-day run-in period. Nasal symptoms (itching, stuffiness/congestion, rhinorrhoea, and sneezing) and non-nasal symptoms (itching or burning eyes, itching of ears or palate, eye redness, and tearing) reflective of the previous 12 h were scored in the morning and the evening. The primary efficacy assessment was the mean change from baseline in the total symptom score (TSS) averaged over the 2-week study period. The 28% (spring) and 30% (autumn) reduction in the TSS produced by desloratadine was significantly greater than the reduction produced by placebo (13% ($P < 0.01$) and 22% ($P = 0.02$), respectively) (Fig. 3). Similar results were obtained in the treatment of perennial allergic rhinitis, in which the 4-week average change in instantaneous TSS showed a significantly greater reduction from baseline with desloratadine treatment than with placebo ($P = 0.005$) (Fig. 4) (62).

In patients with SAR and mild-to-moderate asthma, desloratadine relieves symptoms of both conditions. Two multicentre, randomized, double-blind, placebo-controlled, parallel-group investigations were conducted in a total of 613 patients aged 15 years or older who had a minimum 2-year history of SAR and concomitant mild-to-moderate asthma that worsened during the autumn/winter allergy season (63). Participants had to have a prespecified severity of symptoms of SAR and asthma for 3 days

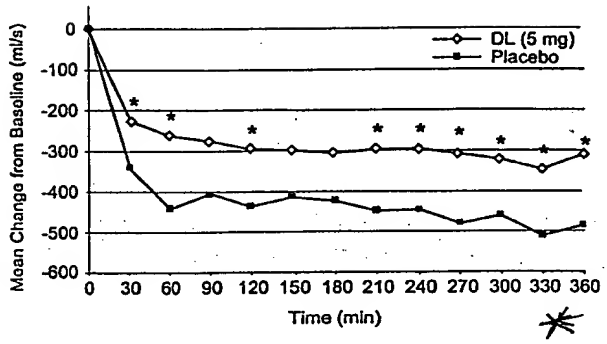

Figure 6 Mean change (decrease) from baseline nasal airflow (in ml/s) in response to allergen exposure after treatment with desloratadine 5 mg (DL) or placebo every morning for 7 days. *P* values refer to comparison between desloratadine and placebo. $^{*}P = 0.005$. From (67).

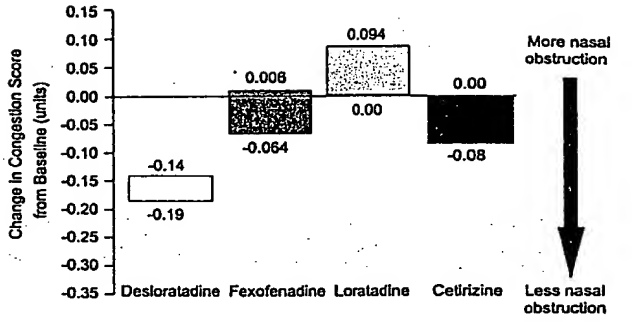

Figure 7 Systematic review of double-blind, placebo-controlled studies that examined the activity of second-generation histamine H_1-receptor antagonists on nasal obstruction. Adapted from (68).

and nasal and non-nasal SAR symptom severity. Nasal obstruction was documented objectively as nasal airflow (mL/s), measured by active anterior rhinomanometry immediately before (baseline) and every 30 min during allergen exposure.

Desloratadine administration was associated with less decrease from baseline, as compared with placebo, in the mean nasal airflow at most assessment times (primary endpoint) and in the mean severity score for the symptom of nasal obstruction at all assessment times. Desloratadine administration was associated with significantly less severely decreased nasal airflow within 30 min of allergen exposure ($P < 0.02$), a benefit that continued throughout the 6-h period of allergen exposure (Fig. 6). With desloratadine, nasal secretions were also less ($P < 0.001$), and symptoms were less severe, including nasal congestion ($P < 0.002$), rhinorrhoea, and sneezing (67).

A systematic evaluation of double-blind, placebo-controlled studies assessed the effects of cetirizine, fexofenadine, loratadine, and desloratadine on nasal obstruction (68). Only studies that used clinically approved drug doses and specifically reported effects on nasal obstruction (congestion) were included. Placebo effects were factored out, and severity scores were standardized. Desloratadine was the only agent that consistently reduced nasal congestion greater than placebo in all studies analysed (Fig. 7).

Safety

Desloratadine is tolerated well (65). In controlled clinical studies, the rate of reported anticholinergic and sedative effects was similar to that with placebo. Also, there were no clinically significant abnormalities in electrocardiographic parameters, laboratory profiles, or vital signs.

Improved quality-of-life measures

Given the burden of allergic diseases on psychosocial well being, one goal of treatment should be the improvement of quality of life. Desloratadine improves quality of life by decreasing the effect of allergic symptoms on sleep and daily activities. A multicentre, randomized, double-blind, placebo-controlled study of 190 patients with CIU of at least 6 weeks' duration determined that desloratadine 5 mg once daily improved both CIU-impaired sleep and CIU-impaired ability to conduct daily activities – an effect that was evident after the first dose and was maintained throughout the 6 weeks of the study ($P < 0.05$) (60). In a German postmarketing study of nearly 50 000 patients, SAR caused moderate or severe interference with daily activities in 40% of patients before desloratadine therapy, compared with only 3% during therapy. Similarly, before therapy 34.3% of patients experienced moderate or severe disruption of sleep, compared with only 2.6% during therapy (57).

Conclusions

Scientific, genetic, epidemiological, and pharmacoeconomic research has transformed our understanding of allergy. New endpoints for research and new therapeutic targets have become clear. Efficacy endpoints of antiallergy treatment are also changing. Ideally, new antiallergy therapies should intervene in the systemic allergic inflammatory cascade, with broad clinical efficacy in multiple allergic disease states, an improved clinical efficacy profile compared with existing therapies, and a positive impact on disease-impaired quality-of-life measures.

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Original article

Effects of budesonide and fluticasone propionate in a placebo-controlled study on symptoms and quality of life in seasonal allergic rhinitis

Background: Intranasal glucocorticosteroids are effective in seasonal allergic rhinitis. This study compared the efficacy of budesonide (Rhinocort ®Turbuhaler®) and fluticasone propionate (Flixonase®) in this respect.
Methods: Patients ($n=280$) were randomized to receive budesonide, 140 μg (delivered dose) once daily, fluticasone, 200 μg once daily, or matching placebos for 5 weeks. The primary efficacy variable was the change in combined nasal symptom (nasal blockage, runny nose, sneezing) scores. Quality of life was measured in 121 patients by means of the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) and the Short-form Health Survey (SF-36).
Results: Both steroids significantly reduced combined nasal symptoms, compared with placebo. There was no significant difference between the two treatments. Substantial or total symptom control was achieved in 89.9% of the budesonide-treated patients, compared with 88.7% with fluticasone and 42.7% with placebo. Four of the five domains of the RQLQ were significantly improved with budesonide, whereas with fluticasone only two domains were improved. Budesonide significantly improved scores in five out of eight domains of the SF-36, whereas no domains were improved with fluticasone.
Conclusion: There was no significant difference in efficacy between budesonide and fluticasone in this study. However, greater improvements in quality of life were seen with budesonide than with fluticasone.

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Key words: budesonide; clinical trial; fluticasone; quality of life; seasonal allergic rhinitis.

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Seasonal allergic rhinitis is very common and in addition to the classic rhinitis symptoms of nasal blockage, rhinorrhea, itching, and sneezing, patients have an impaired quality of life (1). The Rhinitis Quality-Of-Life questionnaire (RQLQ) has been used in several trials testing the effect of nasal glucocorticosteroids in seasonal allergic rhinitis (2–6). In these studies, QOL was found to be improved by treatment.

The treatment of seasonal allergic rhinitis is mainly based on antihistamines and topical glucocorticosteroids (1). Budesonide is a glucocorticosteroid that has been used for several years for the topical treatment of asthma and rhinitis, and has been shown to be effective and safe (7). The dry powder formulation (Rhinocort Turbuhaler®) of budesonide, which consists of the active substance only, has been shown to be effective and safe in the treatment of grass pollen-induced allergic rhinitis at metered doses of 200 μg and 400 μg taken once daily (8). The corresponding delivered doses from this device are about 70% of the metered (9), i.e. approximately 140 μg and 280 μg.

Fluticasone propionate, another glucocorticosteroid, is available for topical nasal use in an aqueous formulation, at a recommended dose of 200 μg, given once daily (10,11).

In a study of patients with perennial allergic rhinitis, intranasal budesonide 140 μg and 280 μg (delivered doses) and fluticasone propionate 200 μg were compared. No significant differences were seen between the active treatments while all three were significantly more effective than placebo (12).

The aim of the present study was to compare the efficacy of 140 μg once daily budesonide dry powder with 200 μg once daily fluticasone propionate aqueous suspension in patients with seasonal allergic rhinitis by means of using combined and individual nasal symptom scores and HRQL assessments.

Material and methods

Study design

There was no blinding between treatments but the active and placebo treatments were blinded with respect to each other by the use of a masking device; the budesonide treatment (Turbuhaler®) differed in

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appearance from the fluticasone propionate treatment (aqueous spray). Patients were assigned to parallel treatment groups according to a computer-generated block randomization list.

The study involved 10 centres in France and 9 in Italy. The HRQL assessment was performed in the French centres only. The study comprised a 1-week screening period, a 1-week run-in period, and a 5-week treatment period.

Patients

Patients with an age range from 18 to 69 years, with a history of seasonal rhinitis for at least 2 years and allergic to grass and/or *Parietaria* pollens were studied. Pollen allergy was verified by positive skin-prick test (SPT), RAST or CAP system tests. Only patients with moderate to severe rhinitis were included. They had to have symptoms requiring at least antihistamine treatment during the previous pollen season and to have at least two nasal symptoms (blocked nose, runny nose or sneezing) recorded during the run-in period. Patients were excluded if they had concomitant perennial allergic rhinitis, signs of active infection, or structural abnormalities in the nose. Pregnant or breast-feeding women were likewise excluded. No topical, systemic or depot steroid treatment was permitted within the 6 weeks before the run-in period. Antihistamines had to be stopped within 48 h (astemizole 8 weeks) prior to visit 1. Each patient gave written consent to participate. The study was approved by the Local Ethics Committees and was performed in accordance with the principles stated in the Declaration of Helsinki.

Treatment

During the screening, run-in and treatment periods, patients were only allowed to use antihistamine tablets (cetirizine, 10 mg OD) and eye drops (disodium cromoglycate) as rescue medications. Patients eligible for inclusion were then randomized to one out of four treatments for 5 weeks: budesonide 140 µg delivered dose (Rhinocort Turbuhaler® 100 µg metered dose, AstraZeneca, Lund, Sweden: one inhalation in each nostril every morning); fluticasone propionate 200 µg (Flixonase® 50 µg, GlaxoSmithKline, Uxbridge, UK: 2 sprays in each nostril every morning); placebo budesonide Turbuhaler® or placebo fluticasone propionate (administered in the same fashion as the active treatments). No concomitant medication, apart from the rescue medications, was allowed during the study.

Assessments

All patients recorded daily symptoms on a diary card throughout the study period. Nasal symptoms (blocked nose, runny nose, sneezing), as well as eye symptoms, were assessed every evening and scored as follows: 0=no symptoms; 1 = mild symptoms, not troublesome; 2 = moderate symptoms, frequently troublesome but not enough to interfere with normal daily activity or night-time sleep; 3 = severe symptoms, troublesome enough to interfere with normal daily activity or night-time sleep. Intake of study medication and the use of antihistamine rescue medication were also recorded in the diary.

At clinic visits at the start, and after 2 and 5 weeks of the treatment, information on adverse events was collected by means of a standard question: 'Have you had any health problems or symptoms not usually associated with your seasonal allergic rhinitis since your last visit?', and a nasal examination was performed. At the end of the treatment period, patients were asked to make an overall evaluation of the treatment efficacy according to a 5-grade scale, where 0 = aggravated symptoms and 1, 2, 3 and 4 referred to no, minor, substantial and total control of symptoms, respectively.

Quality of Life assessment

Patients' HRQL was assessed in the French centres, using three questionnaires; the specific rhinitis questionnaire, the Rhinoconjunctivitis Quality of Life Questionnaire (RQLQ) (13), the generic health status questionnaire, and the Medical Outcome Study Short-Form Health Survey (SF-36) questionnaire (14), all of which were administered at the end of run-in and again after the treatment period. The questions referred to the week prior to the visit at the clinic, and patients were required to mark their responses on an ordinal scale or to answer 'yes/no'.

The RQLQ has 24 items which are divided into six domains: activities (three items), sleep (three items), general problems (seven items), practical problems (three items), nose problems (four items) and emotional state (four items). The SF-36 questionnaire is based on 36 items selected to represent nine health concepts (physical and social functioning, role limitations in physical and emotional problems, mental health, vitality, bodily pain, general, general perception of health, change of health).

Pollen counts

Pollen counts were recorded for all centres in a standardized way throughout the study period and it was found that grass and/or *Parietaria* pollen were present during the period of the study.

Statistical analysis

The primary efficacy variable was the combined nasal symptom score (the sum of the three individual nasal symptom scores). The mean value of this variable, as well as the mean values of individual symptom scores over the 1-week run-in and the 5-week treatment period, were calculated. Changes from the baseline in the mean combined nasal symptom scores were compared using ANOVA, with treatment, center and baseline score factors as covariates in the model, followed by pairwise comparisons.

ANOVA was also used for the analyses of the secondary efficacy variables: changes in individual nasal symptom scores; patients' overall evaluation of treatment efficacy at the end of the study, and the use of cetirizine as a rescue medication.

The HRQL questionnaires were analyzed using the change from the beginning to the end of treatment. Changes were compared between treatments using an ANOVA model, which included the factors treatment, center, and baseline QoL scores as covariates.

Compliance was assessed as the number of doses marked as taken in the diaries relative to the number of doses according to the protocol and the proportion was analyzed using the Kruskal–Wallis test.

In all tests of significance two-tailed alternatives were used. $P < 0.05$ was considered statistically significant.

Table 1. Baseline characteristics for all patients treated

	BUD (n=104)	FP (n=102)	Placebo (n=101)	All (n=307)
Age mean (years)	34.3	34.3	34.9	34.5
Range	17–59	18–69	18–64	17–69
Weight mean (kg)	66.7	65.9	66.6	66.4
Range	45–113	47–90	43–96	43–113
Height mean (cm)	169	169	169	169
Range	148–190	150–194	148–192	148–194
Rhinitis Duration mean (no. of years)	11.6	10.5	11.5	11.2
Range	1–34	1–48	1–50	1–50

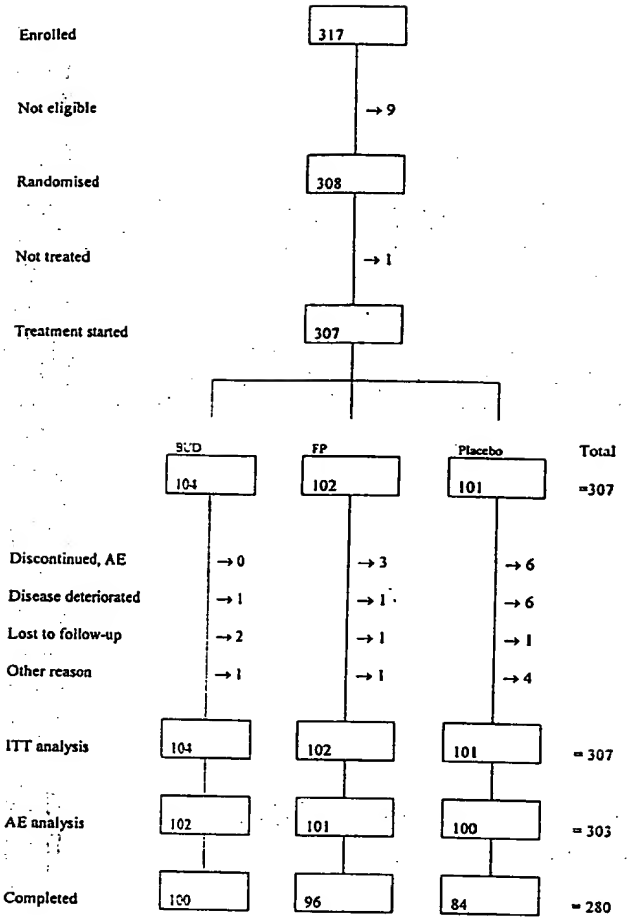

Figure 1. Patient distribution.

Results

Patient population

A total of 308 patients were randomized, 307 (152 males, 155 females) were treated (one patient was lost to follow up), and 280 patients completed the 5-week study. For the HRQL assessments (at the French centres only) data from 121 patients were available. The treatment groups were comparable as regards sex distribution, age and duration of disease (Table 1).

Clinical efficacy

A total of 307 patients were evaluated and included in the Intention To Treat (ITT) analysis (Fig. 1). The mean baseline period was 7.2 days (range 3–23) and the mean treatment period was 35 days (range 1–58).

Combined nasal symptoms In the two placebo groups, the combined nasal symptom scores were only minimally changed from baseline values and were very similar (Fig. 2). Hence, the two placebo groups were combined for statistical analyses. In the two actively

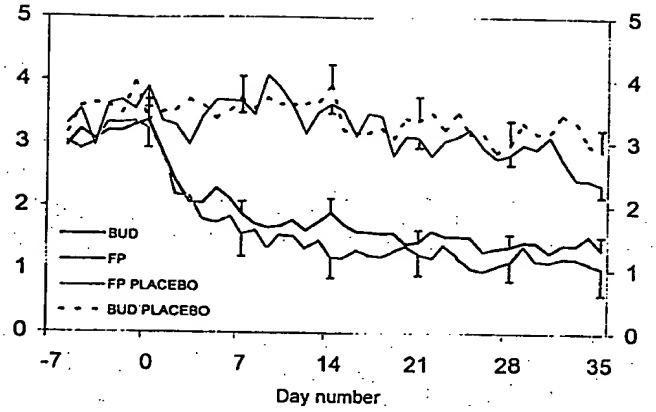

Figure 2. Daily means incl S.E.M of Combined Nasal Symptoms by treatment during run-in (days −6–0) and treatment period (days 1–35).

treated groups, the total symptom score were reduced within the first week and then remained low for the next 4 weeks. Adjusted mean change from baseline was −1.44 and −1.71 for budesonide and fluticasone propionate vs. placebo, respectively. No significant difference in the combined nasal symptom score could be detected between budesonide and fluticasone propionate ($P=0.11$).

Individual nasal symptoms Scores for each separate nasal symptom significantly decreased in the two active treatment groups as compared with the placebo (Table 2). No statistically significant difference between the two active treatment groups was found for individual nasal symptom scores except for nasal blockage ($P=0.039$).

Eye symptoms Scores for eye symptoms significantly decreased in the budesonide treatment group compared with placebo, but not in the fluticasone propionate treatment group. No significant difference between the two active treatment groups was found for eye symptom scores (Table 3).

Rescue medication The baseline consumption of antihistamine rescue medication was low (mean 2.58, 2.78 and 2.86 tablets per week in the budesonide, fluticasone propionate and placebo-treated groups, respectively). Consumption decreased significantly in the two active treatment groups by comparison with placebo. However, the budesonide-treated patients consumed significantly fewer antihistamine tablets (mean 1.23 tablets/week) compared with the fluticasone propionate group (mean 1.93 tablets/week) during the treatment period ($P=0.030$).

Patients' overall evaluation of treatment efficacy The overall assessment of treatment efficacy made by the

Table 2. Change from baseline of individual nasal symptoms

Blocked nose	N	Baseline mean change	Adjusted mean change	95% conf. limits	P-value
Change from baseline					
BUD	102	1.05	−0.47	−0.56−0.37	
FP	101	1.08	−0.61	−0.71−0.51	
Placebo					
Treatment comparison	100	1.22	−0.02	−0.12 0.08	
BUD vs. FP		0.14	0.01	0.27	0.039
BUD vs. placebo		−0.45	−0.59	−0.31	<0.001
FP vs. placebo		−0.59	−0.73	−0.46	<0.001
Runny nose					
Change from baseline					
BUD	102	1.12	−0.50	−0.59−0.40	
FP	101	1.04	−0.53	−0.68−0.49	
Placebo					
Treatment comparison	100	1.20	0.02	−0.08 0.11	
BUD vs. FP		0.09	−0.04	0.22	0.19
BUD vs. placebo		−0.51	−0.65	−0.38	<0.001
FP vs. placebo		−0.60	−0.73	−0.47	<0.001
Sneezing					
Change from baseline					
BUD	102	1.04	−0.53	−0.62−0.45	
FP	101	1.05	−0.53	−0.67−0.50	
Placebo					
Treatment comparison		1.12	−0.04	−0.12 0.04	
BUD vs. FP	100	0.05	−0.07	0.17	0.39
BUD vs. placebo		−0.49	−0.61	−0.38	<0.001
FP vs. placebo		−0.54	−0.65	−0.43	<0.001

Table 3. Change from baseline of eye symptoms

Eye symptoms	N	Baseline mean change	Adjusted mean change	95% conf. limits	P-value
Change from baseline					
BUD	102	0.69	−0.22	−0.31−0.14	
FP	101	0.66	−0.17	−0.26−0.08	
Placebo	100	0.77	−0.06	−0.15 0.03	
Treatment comparison					
BUD vs. FP			−0.05	−0.17	0.07	0.41
BUD vs. placebo			−0.16	−0.28	−0.04	0.009
FP vs. placebo			−0.11	−0.23	0.01	0.075

patients at the last visit showed significantly better results in the two active treatment groups than in the placebo group ($P<0.001$), with no significant difference between budesonide and fluticasone (Fig. 3).

Compliance with study medication Compliance was 92.6% in the budesonide group, 96.0% in the fluticasone group, and 96.6% in the placebo group, with no significant difference between the two active treatment groups ($P=0.097$).

Health-related quality of life Mean baseline scores for the RQLQ and the SF-36 are summarized, together with changes between baseline and end of treatment.

RQLQ Four out of the five domains, i.e., sleep problems, nonhayfever problems, practical problems and nasal symptoms, were significantly improved following budesonide treatment compared with placebo (sleep; $P=0.009$, nonhayfever, practical, and nasal symptoms; $P<0.001$). The fifth dimension, emotional problems, also showed some improvement, but the result was not significant. Only two out of the five domains (practical problems and nasal symptoms) were improved following fluticasone propionate treatment compared with placebo ($P=0.003$). The two active treatment groups did not differ significantly from each other.

SF-36 questionnaire Five out of the eight domains relating to general quality of life, i.e., physical functioning, vitality, social functioning, role-emotional and mental health, were significantly improved following budesonide treatment, compared with placebo, but there were no significant changes following fluticasone propionate treatment. The two active treatment groups did not differ significantly from each other (Table 4).

The aggregated summary scores Physical Component Summary (PCS) and Mental Component Summary (MCS) are presented in Table 4. The two active treatment groups did not show any significant difference compared with placebo for PCS. For the MCS only the budesonide group showed significant improvements ($P<0.05$) compared with placebo. No significant difference between the two active treatment groups were seen.

Safety

Adverse events From the 307 patients who received study medication, four did not return for any clinic visit, thus 303 patients were eligible for the safety

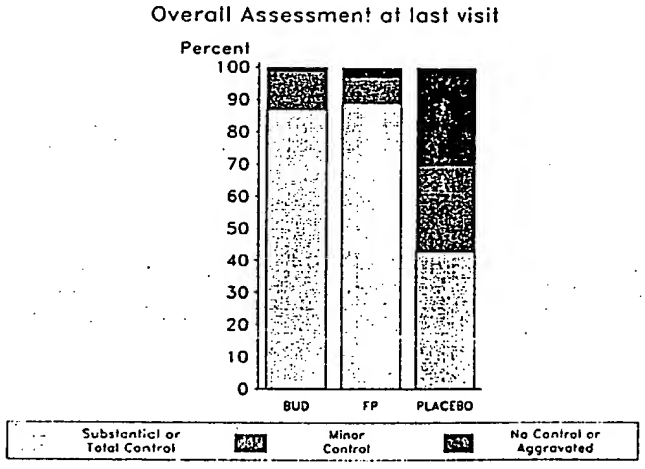

Figure 3. Overall assessment of treatment efficacy at the end of the study.

Table 4. Quality of Life dimensions at baseline and changes from baseline to end of treatment

	BUD		FP		Placebo	
RQLQ Questionnaire	Baseline $n=39$	Change	Baseline $n=40$	Change	Baseline $n=37$	Change
Sleep problems	1.62	−0.99*	1.48	−0.74	1.53	−0.26
Non hay fever symptoms	1.52	−0.90**	1.26	−0.53	1.27	−0.11
Practical problems	3.24	−1.94**	2.95	−1.68*	2.96	−0.73
Nasal problems	3.01	−1.78**	2.65	−1.33*	2.70	−0.53
Emotional problems	2.12	−0.62	1.69	−0.36	1.82	−0.28
SF-36 Questionnaire	$n=41$		$n=42$		$n=38$	
Physical functioning	88.7	4.6*	95.5	0.5	92.9	−0.7
Role physical	80.5	5.9	84.1	7.4	88.2	0.8
Bodily pain	79.0	3.6	83.0	4.6	82.8	−1.1
General health	70.1	1.1	79.0	1.1	78.8	−2.7
Vitality	58.0	6.8*	60.4	5.1	64.1	0.9
Social functioning	67.3	8.3*	72.1	5.1	72.4	0.2
Role emotional	79.2	8.4*	85.4	4.3	87.4	−3.9
Mental health	65.0	7.8*	70.0	4.5	72.7	0.3
Physical Component Summary (PCS)	52.2	0.7	54.5	0.7	54.2	−0.6
Mental Component Summary (MCS)	44.2	4.6**	46.5	2.5	48.0	−0.4

*$P<0.05$; ** $P<0.001$.

analysis. Adverse events were mild and reported by 15% of the patients who were treated with budesonide, 12% treated with fluticasone propionate, and 15% treated with placebo. Only one patient in the budesonide group and two in the fluticasone group complained of episodes of blood-tinged nasal discharge.

Discussion

In the present study we found that the two nasal glucocorticosteroids were equally effective against nasal symptoms of seasonal allergic rhinitis. General quality of life was improved by budesonide but not by the fluticasone propionate. For disease-specific quality of life budesonide improved twice as many domains as fluticasone proprionate. For the primary efficacy variable, the combined nasal symptom score, no significant difference between the two active treatment groups was found. A 0.14 score step difference for nasal blockage was found to be significantly different when comparing fluticasone propionate with budesonide. Not only the doses, but also the presentations (aqueous spray vs. dry powder inhaler) differed between the treatments. There were no significant differences concerning nasal symptom scores between the respective placebo groups, indicating that differences in formulation were unlikely to have influenced the result. The results of the present study in seasonal allergic rhinitis are in line with a similar comparison in perennial allergic rhinitis (12), in which intranasal budesonide dry powder 140 μg and fluticasone propionate aqueous suspension at a higher dose of 200 μg had similar clinical efficacy. The primary efficacy variable, the combined nasal symptom score, decreased during a 5-week treatment, with no significant difference between the two active treatment groups. This study shows that the two nasal corticosteroids are effective in persistent allergic rhinitis induced by pollen. The magnitude of symptom-score reduction (approximately 1.5 score steps) was considered clinically relevant, and all three components of the combined nasal score contributed equally to the reduction.

The clinical relevance of the small difference in scores for blocked nose between the two active treatment groups is, however, uncertain.

As seen in Fig. 2, the efficacy of the active treatments tend to discriminate mean scores from placebo from the first day. During the second week of treatment the active treatments reach a mean separation to placebo, which is not superseded later in the 5-week period.

With regard to health-related quality of life, this study further showed that both generic and disease-specific HRQL are impaired in patients with seasonal allergic rhinitis and can be improved by suitable therapy. The Quality of Life assessment indicated budesonide 140 μg (delivered dose) to be clearly superior to placebo; four out of the five disease-specific domains of the RQLQ and five out of the eight general domains of the SF-36 questionnaire were significantly improved with budesonide. In contrast, fluticasone propionate 200 μg significantly improved only two out of the five disease-specific domains and was not statistically significantly superior in any domain in the generic SF36 questionnaire. In view of the burden allergic rhinitis creates not

only for the patient, but also for the national economy in terms of medical care and lost productivity (15), it is important to consider these wider implications of drug efficacy on patient well-being.

All statistical findings were well above the 0.5 score step difference vs. placebo that has been suggested to be the minimal clinical relevant change for RQLQ (16).

In conclusion, the efficacy of topical budesonide dry powder 140 μg taken once daily, was not significantly different to fluticasone propionate aqueous suspension 200 μg taken once daily, for the primary efficacy variable in seasonal allergic rhinitis. Improvement in general and disease-specific HRQL was significantly superior to placebo in the budesonide group. Both active treatments were well tolerated.

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SUPPLEMENT

The therapeutic index of antihistamines

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Comparing the H_1 profile of second-generation antihistamines

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Introduction

For many years, oral antihistamines have been first-line medications in the treatment of allergic hypersensitivity reactions including allergic rhinitis, rhinoconjunctivitis, urticaria, and pruritus. Most patients usually expect their medication to give fast symptomatic relief from their allergies without undesirable side-effects. Although a number of mast-cell-derived mediators, including histamine, prostaglandins, and leukotrienes, are released during the anaphylactic response, the relief of symptoms given by antihistamine use indicates a major role for histamine in these reactions.

Assessment of the efficacy of the H_1-blocking drugs necessitates the use of both clinical and pharmacoamic studies designed to compare cutaneous or nasal potency. The efficacy of an H_1-receptor antagonist may be assessed, firstly, by measuring inhibition after histamine provocation in the skin and/or the nose, a method which allows the evaluation of potential differences in drug distribution at different tissue sites. Secondly, we can assess the ability to block the response to allergen provocation in the skin, i.e., inhibition of the wheal and flare response; this approach provides information on factors such as the antigen-induced release of other mediators and also on the role that histamine plays in the symptoms of different individuals.

A number of pharmacodynamic study designs are used to compare anti-H_1 antagonist profiles. Single-dose studies provide information relating to the onset, potency, and duration of action of a drug and are the most widely used method of comparing antihistamine potency. A less common approach involves the use of repeated dosage studies; these provide more information on absorption and metabolism of the drug and on its possible accumulation in tissues. The latter design may provide a better clinical prediction of drug efficacy.

This review is divided into three parts: the first will compare anti-H_1 profiles in skin studies; the second will discuss those studies performed in the nose; and the third will compare the effect of one antihistamine, cetirizine, in both the skin and the nose. Finally, the validity of pharmacodynamic studies as predictors of the clinical efficacy of antihistamines will be examined. It is hoped that this approach will stimulate debate about study design, and that this, in turn, may further our understanding of the validity of anti-H_1 profiles as predictors of clinical efficacy.

Pharmacodynamic studies in the skin

A reliable and reproducible method for measuring the effectiveness, onset, and duration of action of an antihistamine is to assess its ability to block the *in vitro* histamine-induced wheal and flare reaction in the skin. Histamine can be either injected intradermally or administered by skin prick test. Study design may involve either an hourly evaluation of the onset and duration of action of each drug at single or various doses (1) or a histamine concentration response at a given time point after drug intake (2, 3).

Time course of efficacy

The former design facilitates assessment of anti-H_1 activity in the 24 h after a single dosing; since the histamine concentration administered in the skin is

histamine to the nasal mucosa. This partially mimics the response to antigen and in particular induces nasal blockage; this can be objectively measured by a technique called posterior rhinomanometry, which assesses the nasal airway resistance by both nostrils simultaneously.

Dose responses to histamine

We first carried out a double-blind, randomized, crossover, placebo-controlled study designed to assess objectively the nasal antihistamine effect of cetirizine in patients with allergic rhinitis and in control subjects (10). The study evaluated the inhibition of the histamine-induced increase in nasal airway resistance after a 3-day treatment with cetirizine 20 mg daily. Nasal challenge was performed by nebulization of incremental double doses of histamine (0; 0.04–1.28 mg/nostril) in six patients with allergic rhinitis and six control subjects taking either cetirizine or placebo. In both control subjects and patients with allergic rhinitis, cetirizine treatment resulted in a significant reduction in nasal airway resistance and perceived sensation of nasal obstruction, after dose-dependent administration of nebulized histamine (10).

Time dependence of efficacy

Posterior rhinomanometry was also used to compare the efficacy of cetirizine, loratadine, and placebo in terms of their onset and duration of action in double-blind, cross-over, placebo-controlled studies performed in patients with allergic rhinitis (11, 12). Patients who were asymptomatic at the time of the study took the single therapeutic dosage of 10 mg in each case. We assessed the maximal effects of cetirizine and loratadine at 4 h, their early effects at 1.5 h, and their late effects at 24 h. Since histamine is subjected to tachyphylaxis in the nose, the subjects were required to undergo posterior rhinomanometry on three separate occasions separated by at least 4 days: one assessed the effect 1.5 h after drug intake; another the effect at 4 h; and another at 24 h. Histamine dose responses were applied to the nasal mucosa in both nostrils, and posterior rhinomanometry was carried out at 0 (saline), 1.5, 3, 5, and 7.5 min after each nebulization. We observed a significant decrease of histamine-induced nasal airway resistance 4 h after intake; this was the maximal drug effect and was similar for both cetirizine and loratadine. The baseline was similar for all patients, and it was noted that the increase was not entirely blocked with cetirizine, as it was in the skin. At 1.5 h, histamine-induced nasal airway resistance was significantly inhibited by both cetirizine and loratadine, an effect which decreased with increasing histamine concentrations. Cetirizine was more active than loratadine, a finding which is in agreement with those obtained in the skin, and which suggests that cetirizine has a more rapid onset of action. At 24 h, loratadine did not significantly inhibit histamine-induced nasal airway resistance compared with placebo, although there was a trend toward inhibition. In contrast, the study by Simons et al. (1) demonstrated that loratadine was still active in the skin 24 h after administration, whereas, in our study, only cetirizine had a significant effect on histamine-induced nasal airway resistance compared with placebo at the later time point. These data are summarized in Fig. 2.

The difference in the duration of the nasal action between cetirizine and loratadine might be explained by differences in their metabolism. Loratadine is rapidly metabolized to its therapeutically active form in the liver, whereas, as cetirizine does not require biotransformation, it is active immediately after absorption, being eliminated as unchanged drug. The discrepancy in the effect of loratadine at 24 h between the skin (1) and the nose (12) suggests that these types of pharmacodynamic studies should be performed at both sites, since biodistribution of the drug may differ between these organs.

Comparative study in skin and nose

In order to assess the potential effects of biodistribution in different tissues, the efficacy of cetirizine was compared in the skin and nose of patients with allergic rhinitis. Cetirizine was chosen because it is the most powerful antihistamine available in terms of suppression of histamine-induced symptoms, and it is directly active and unmetabolized. The rationale was similar to that of Wood-Baker & Holgate's study (13) in which the inhibition of bronchospasm after histamine inhalation was compared with cutaneous inhibition by a large panel of antihistamines. The results demonstrated a good correlation between the protective effect of antihistamines both in the lower airways and in the

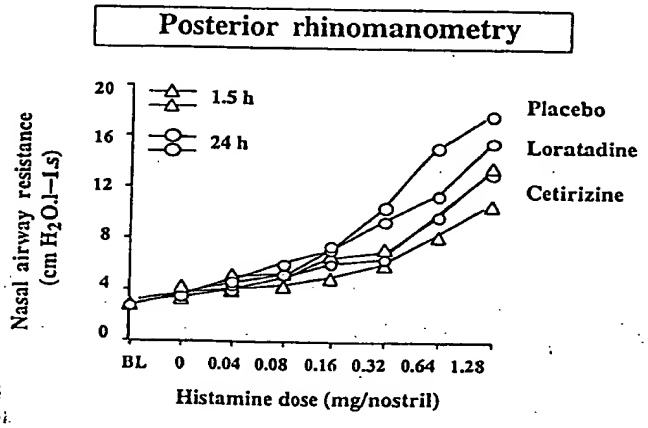

Figure 2. Effect of treatment with loratadine and cetirizine compared with placebo at 1.5 and 24 h on nasal airway resistance in volunteers skin-prick-tested with increasing concentrations of histamine (0, 5, 10, 50, 100, 200, and 300 mg/ml) followed by drug administration.

skin. We reasoned that comparison of the nasal and cutaneous activity of cetirizine would yield information on the intraindividual biodistribution of the drug.

The study was a randomized, double-blind, cross-over, placebo-controlled trial carried out over 2 months; at inclusion, subjects underwent nasal allergen provocation; 4 weeks later, they attended on 2 days separated by 1 week to undergo cutaneous or nasal histamine provocation 4 h after intake of either cetirizine or placebo. One week later, they attended on two different days separated by 1 week for nasal or skin provocation, respectively. We observed 97% inhibition by cetirizine of the histamine-induced wheal area compared to placebo and good inhibition of the nasal response, demonstrating a strongly positive effect for the drug in both organs. However, no correlation was observed between the cutaneous and nasal activities, a finding which might be explained by the fact that the inhibitory effect was almost total in the skin and only partial in the nose (Frossard et al., paper submitted). It may also be a consequence of the additional irritative effect associated with nasal provocation, something which is not seen with skin prick testing. It may be possible to improve the correlation coefficient by reducing the cetirizine dosage given in the cutaneous section of future comparative studies. Further pharmacodynamic assessment of the relative bioavailability of drugs between the two organs should improve our ability to predict the clinical efficacy of an antihistamine in both cutaneous and nasal allergic disorders.

Nasal provocation with allergen might provide additional information when assessing the potency of antihistamines and thus could be included in the pharmacoclinical group of comparative studies. In particular, informative data on the nasal efficacy of a drug could be obtained from objective measurement of airway reactivity and inflammatory parameters modified by allergen administered to the nasal mucosa at provocative or low realistic doses. This approach could, for example, include the objective measurement of nasal airway responsiveness by rhinomanometry before and after allergen exposure. The associated inflammatory events in the nasal lavage fluid could be measured in a manner analogous to recent studies that measured eosinophil cationic protein in bronchial lavage fluid before and after allergen exposure at low subclinical doses (14) and the inflammatory neuroimmune factor, nerve growth factor (15).

Conclusion

Comparison of the H_1 profile of second-generation antihistamines by pharmacodynamic studies permits a degree of objective assessment when rating the clinical efficacy of different drugs. Methodologies should involve multiple steps and include single-dose studies which facilitate assessment of efficacy, onset and duration of action, and the consistency of interindividual efficacy. Addition of repeated dosages to this model would enhance the assessment by allowing measurement of efficacy not only at steady state but also under conditions of dynamic equilibrium, a procedure which could take account of factors such as the absorption and metabolic clearance rates of the drugs. Although Monroe et al. (16) have stated that there is not necessarily a correlation between what is observed in the skin and what is observed clinically, these types of studies nonetheless provide important information on the relative potencies of the second-generation compounds.

Discussion

Donnelly: Monroe et al. (16) report that there is not necessarily a correlation between the efficacy of an antihistamine in the skin and the clinical efficacy. The remark has to be made that if no difference emerges from a clinical study between two compounds, it does not necessarily mean that the two compounds are similar. It simply means that the study is not sensitive enough – in other words is underpowered – to detect a clinical difference, even though this may well exist.

Frossard: Indeed, the study might not be sufficiently large to demonstrate a correlation which can be shown in a pharmacodynamic study involving an agonist and its antagonist. A study of this type gives information on the efficacy of the antagonist in terms of onset and duration of action and maximal potency; it also assesses the biodistribution of a drug at different sites. It is important not to extrapolate results of efficacy from one organ site to another. Let's imagine that a good antagonist at receptor binding gives a low pharmacodynamic result in the skin; this will impair the commercialization of the drug if the test is based on that result alone. However, if the inhibitory effect in the nose was good, it would be a good drug to use in the relief of nasal allergic disorders. This subtlety was missing from Monroe et al.'s analysis. Thus, knowledge of the efficacy, onset, and duration of action of a drug at each site should be assessed before assessing the clinical efficacy.

De Vos: In terms of predictability or correlation between skin and nose for H_1 antagonists, the nose model assesses nasal blockage to histamine through posterior rhinomanometry. In real life, nasal obstructions in allergy sufferers are not due only to histamine. In seasonal allergic rhinitis, the problem is mainly sneezing. In this model, you can make a rank order of antihistaminic drugs. So my comment is that when you look at the literature like the Simons study in the skin, you have the rank order of H_1 antagonists' potency.